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Progress Report on New Crops Research

ARS New Crops Meeting
Peoria, Illinois
December 4, 1964

United States
Department of
Agriculture

National Agricultural Library

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GENERAL COMMENTS FROM NU

1. Meetings.

Recommendation of National Coordinating Committee (June 1963)

The National Coordinating Committee on New Crops recognizes that good progress is being made in the preliminary phases of the national program to discover and develop new industrial crops.

The level of Federal support of utilization research on new crops has permitted a reasonable, vigorous and productive program on this aspect of the total problem. However, comparable support has not been extended to the equally essential farm research required if these potential new crops are to attain the ultimate goal of commercial production.

This Committee therefore unanimously recommends to the Agricultural Research Service and to State Experiment Station Directors that immediate steps be taken to develop a mechanism for agronomic and agricultural engineering research on potential new crops which will realistically complement the utilization research program.

Recommendation of S-9 Committee on New Crops

After considering the toxicity of *Cassia occidentalis*, the Committee feels that it can be grown in many areas in the South. The Committee therefore requests that NU undertake the research necessary to develop this crop. This work should include feeding trials with both plant material and seed.

Recommendation of NC-7 Committee on New Crops

The Committee felt that there should be some control over crambe seed for planting. This was so that industry would not take it over and produce it with no credit given to public agencies. They recommended that CR and UR publish an updated revision of the earlier crambe bulletin. The administrative adviser of the Committee suggested a meeting of all agronomists who grew crambe, so all available knowledge might be pooled for inclusion in such a bulletin.

2. Selected items from the oilseed screening and characterization which are called to the group's attention.

Euphorbia lagascae. New source of epoxy acid (58% in oil) in a species having apparently good agronomic prospects.

Crepis. Genus containing members high in epoxy acids (sp. *vesicaria*, 48%) and high in en-ynic acid (*foetida*, *thomsonii*, 36-60%). The en-yne is reactive and can be converted to conjugated trienes and to cyclic acids in good yield.

Cardamine impatiens. One-fourth of acids in seed oil are vicinal dihydroxy saturated long-chain acids (C_{18}-C_{24}).

Helichrysum bracteatum. Oil contains 38% unusual fatty acids including acetylenes, hydroxy acetylenes, epoxides, and dienols. Complex oil.

Calea urticaefolia. Oil contains 31% of previously unknown t-3,c-9,c-12-octadecatrienoic acid.

Echium plantagineum. High iodine value oil containing 13% all cis 6,9,12,15-C_{18}-tetraene. Also other similar borage plants.

Leavenworthia torulosa. Fifty-three percent C_{20} monoene in oil.

3. Commercial acreage for crambe is indicated for 1965. A critical problem receiving emphasis is to ascertain how to obtain a good feed meal by practical procedures.

4. The mucilage-bearing legume seed most outstanding from the utilization standpoint is Cassia javanica which has large seed size, and contains more than one-third water-soluble mucilage.

5. Trial kenaf plantings for captive use in paper manufacture are in progress by two companies. NU efforts are directed toward obtaining data on behavior and processing of the raw material that industry can use as a basis for making specific products or blending pulps as they desire.

SAMPLE PROCUREMENT AND BOTANICAL INVESTIGATIONS

During the period covered by this report (December 1962-August 1964) 1822 seed and whole-plant samples were obtained for new crops screening through the New Crops Research Branch. This figure is not startlingly high or low. It is cause for some concern however when we examine a breakdown of this figure into sources of samples provided. Seven PL 480 projects supplied 1675 of the 1822 samples. Staff botanists of the New Crops Research Branch collected the remaining 147 and more than half of these were collected on field trips having objectives other than samples for new crops screening. This pattern was dictated, of course, by available funds.

Number of Samples from December 1962-August 1964

Israel (November 1965; 1)*	56
Korea (December 1967; 3)	33
Pakistan (January 1967; 2)	247
Spain (February 1966; 1)	271
Turkey (March 1965; 0)	596
Uruguay (May 1966; 2)	226
Yugoslavia (May 1966; 1)	246

*Project expiration date and number of collecting seasons remaining.

The needs of the screening program were adequately filled over the past 2 years. They may be adequately filled for one more year. But after 1 year from now we will have only three PL 480 projects supplying samples for chemical screening. After 2 years from now, only one. New PL 480 projects may be negotiated to replace some of those terminated, but the distribution of so-called surplus currency countries does not coincide with areas which represent new collecting territory for our program. The Mediterranian flora, through the activities of projects in Spain, Yugoslavia, Turkey, and Israel, has been sampled to the extent feasible under PL 480 projects. Duplication of species collected is now becoming a serious problem. Only eastern Turkey offers a good opportunity of supplying species new to the program. We are attempting to negotiate a new, 2-year project for the collection of samples from eastern Turkey. The Mediterranean region could yield many more new samples of interest to new crops screening if such collecting could be undertaken by our own staff botanists who could be expected to avoid duplicating earlier collections.

Another serious weakness in the present arrangements for sample procurement is that they do not provide for sampling the vegetation of the United States. Only in a sporadic, incidental manner have we been able to obtain, in 7 years, a few hundred samples of U.S. plants. Yet from the new crops standpoint our native species offer the best chances of successful adaptation to our crop environments.

PL 480 projects are proving unsatisfactory in another way. For the most part we have been unsuccessful in getting them to undertake intensive collecting of genera in which highly promising leads have been discovered. The type of intensive field work undertaken for Calenduleae, Lesquerella, Cuphea, and Limnanthes by NCRB staff botanists has not materialized for Crepis, Euphorbia, Vernonia, and other exceedingly promising leads from PL 480 countries. We have tried to stimulate the necessary interest to motivate them in field work of this kind but the response has been poor. Our own funds are woefully inadequate for undertaking this important followup work with our own botanists.

If the screening program is to continue at its present level then it is apparent that regular funds will have to absorb more of the sample procurement costs as PL 480 sources dry up. The 1965 collecting season will require two full-time botanists to offset the loss of samples from PL 480 sources. The 1966 season will require four; and from then on NCRB will need five botanists to provide NU with materials for screening and lead evaluation studies.

SEED SAMPLES RECEIVED AND ANALYZED

	Increase since Oct. 31, 1962	Total to Aug. 31, 1964
Seed Samples Received	1948	6063
Families	7	160
Genera	255	1307
Species	1039	3498
Incomplete identification	96	274
Samples Analyzed	1667	5309
>20% oil	491	2053
10-20% oil	324	982
>35% protein	170	659
>60% oil plus protein	173	754
<35% protein plus oil, no starch	317	980
Starch test positive	341	1083
Oil Samples Analyzed		
Isomerization	0	187
GLC	443	1159
Samples Disposed of Without Screening	69	134
Samples Deferred (Four Replicates Analyzed)		80
Other Samples		
Increase samples received	88	402
Increase samples analyzed	72	208

500 additional samples from [illegible]

SEED LIPID SCREENING AND CHARACTERIZATION RESEARCH

I. Information on Assigned Oilseeds

A. Vernonia anthelmintica. No samples of V. anthelmintica have been analyzed this period. V. nitidula (CPR III) was found to have oil with 77-80 percent 18:2 but only 2.5 percent apparent epoxy acid. V. altissima (CPR II) oil contained 72 percent 18:2 and only 1 percent epoxy acid.

B. Dimorphotheca and Lesquerella. No new sources containing significantly large amounts of dimorphecolic acid were encountered although many samples contained a small proportion as evidenced by UV and HBr reaction. Four additional species of Lesquerella were analyzed and found comparable in composition to those reported previously.

C. Cuphea, Limnanthes and petroselinic acid oils. No samples of Cuphea or Limnanthes have been analyzed, nor have we found any comparable materials.

Several methods have been explored for determining petroselinic acid after separation from oleic acid by thin film chromatography. Direct densitometry of charred plates with the equipment we have available has not given the accuracy we need. Measurement of the cis bonds in the UV after TLC of the 18:1 peak trapped from GLC appears feasible but requires purification of the silica and solvents used. The method preferred at present is the separate elution of the petroselinic and oleic spots from TLC, and GLC of each spot with an internal standard. It is planned that Umbelliferae oils will be stripped of essential oils by steam distillation, the fixed oils will be recovered to give an estimate of the volatiles and then analyzed for unsaponifiables and fatty acid composition. Little effort will be expended on the essential oils unless some suggestion of special values is received. A number of Umbelliferae have shown 78-84 percent 18:1 but analysis for petroselinic acid has been completed on only two:

Petroselinum crispum	CPR II	petroselinic 77.8%	oleic 6.2%
Ducrosia anethifolia	I-II	petroselinic 61.6%	oleic 10.9%

D. Erucic acid oils

1. Analyses at Montana State College. The contract has been completed and 600 analyses have been reported. Analyses of 161 samples of Brassica juncea (brown and yellow mustards) showed a range of erucic acid from 18-48 percent, with most of the yellow varieties in the 18-25 range and the brown from 36-42 percent. B. campestris (rape, field mustard) ranged from 17-54 percent erucic (167 samples) with a small group in the 23-30 percent range and the majority in the 34-52 percent range. B. napus (rape) ranged from 26-50 percent erucic acid (43 samples) mostly between 33-40 percent. It is from this species that the Canadian group developed lines with no erucic acid and with 60 percent erucic acid.

Other species found to produce oil richest in erucic acid and not reported in 1963 include the following:

Brassica chinensis	51%	Brassica nigra	44%
Brassica oleracea	49%	Brassica coulorapa	43%
Brassica pervidus	48%	Brassica alba	40%
Brassica hirta	46%	Alyssum alyssoides	40%
		Rapistrum rugosum	40%

2. Analyses at the Swedish Seed Association. The work under this PL-480 Grant is off to a good start because of previous work at the station in the development of inbred lines of rape and mustard. In analyses of 159 individual B. napus plants in the inbred generation under study in the second reporting period (Oct. 1, 1963-Mar. 31, 1964) one was found with 58 percent C_{22} acids. In another population of 132 individuals, several were found with 58 percent C_{22}. In Sinapis alba the high individual samples also showed 58-60 percent C_{22}. In B. campestris, the high individual contained 63 percent erucic acid, higher than any sample encountered in work at NU or Montana. All work has been done on seed from isolation bags and no screening for thioglucosides has been possible. Inbreeding will be continued and crosses will be made.

3. Analyses at NU. Continued screening has not revealed any oils richer in erucic acid than crambe, although oil from Crambe hispanica, a very similar species, is comparable with 55 percent erucic. Two accessions of Sisymbrium alliaria, a species reported in the literature to be richer, were found to contain 42 and 47 percent erucic. One variety of Sinapis arvense (CPR I, thioglucoside 7.2%) produced oil with 54 percent erucic in contrast with the 40 percent reported for the species in 1963. Oil from Erucastrum strigosum (CPR I, thioglucoside 5.9%) contains 49 percent erucic acid and that from E. abyssinicum contains 40 percent. Two samples of the latter species contain 4.7 and 6.9 percent thioglucosides, none of which formed oxazolidinethiones. The demonstrated productivity of E. abyssinicum, the absence of glucosides forming oxazoledinethiones, and the occurrence of a high level of erucic acid in a closely related species suggest the possibility of developing a useful crop. Other high-erucic oils encountered were Brassica sp., 51 percent, and Iberis umbellata, 50 percent.

Relatively few species have been analyzed for total thioglucoside. Those not mentioned elsewhere in this report, and omitting crambe, are tabulated here.

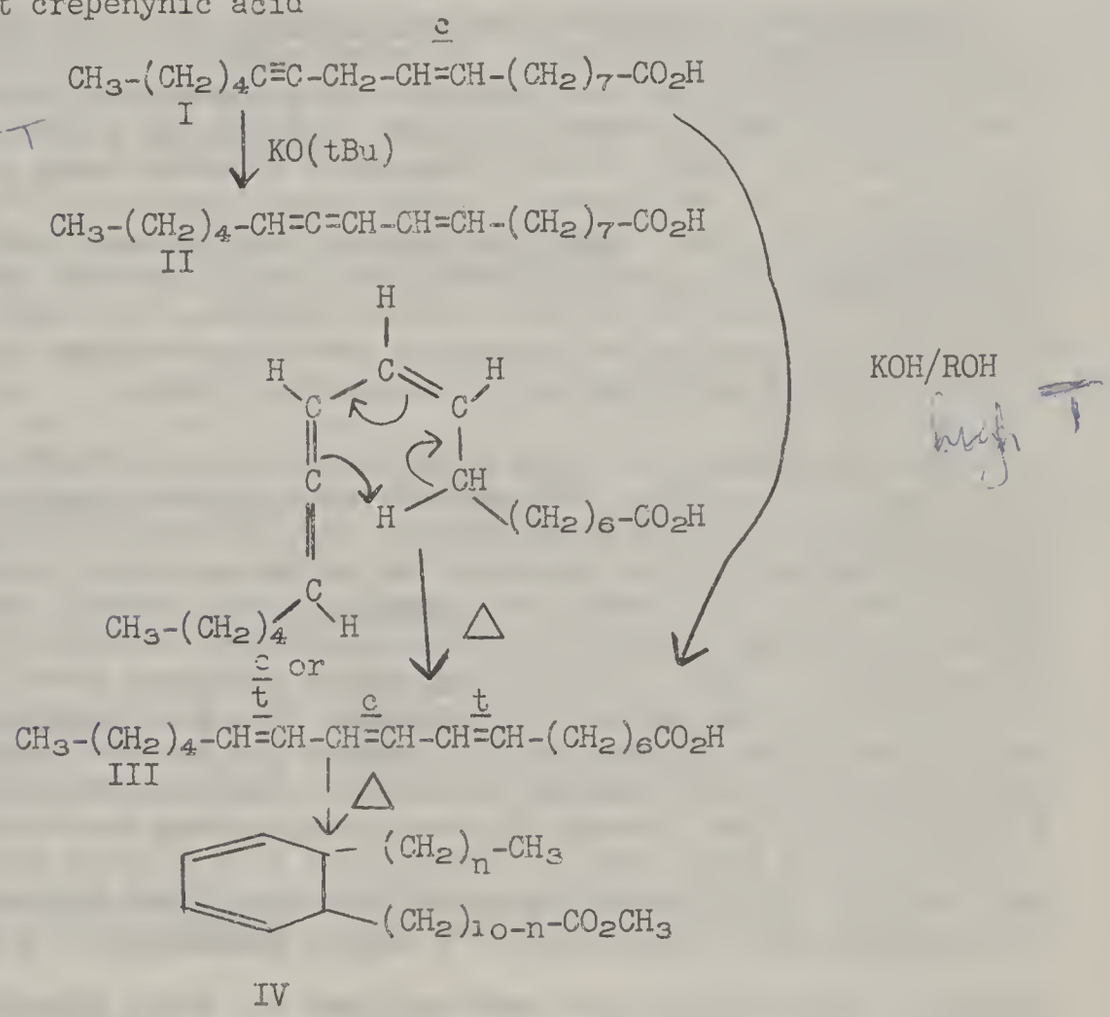
Thioglucoside Content of Oil-Free Air-Dry Meal. Total sulfate method corrected for initial blank.

Brassica juncea	7.2%	Cardamine impatiens	7.0%
Brassica napus	7.2%	Cheiranthus cheiri	10.3%
Sinapis alba	5.8%	Rutabaga	6.2%
Arabidopsis thaliana	3.0%		

II. Organic Characterization Research

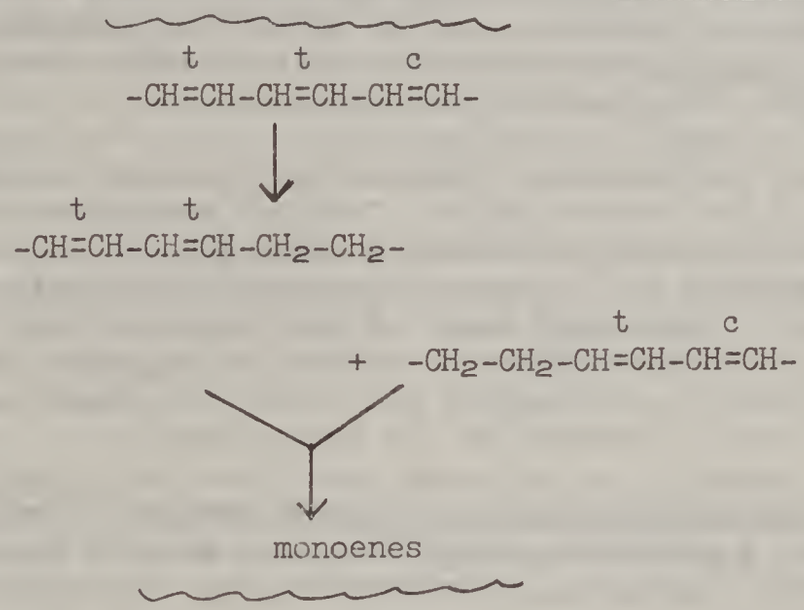
A. Characterization and preliminary developmental work completed.

1. *Crepis foetida* (CPR good) seed oil was shown earlier to contain 60% of an acetylenic analog of linoleic acid which was named crepenynic acid (I). It has been shown that crepenynic acid



rearranges to a conjugated triene when heated with strong alkali, and that this triene can be converted to a cyclic acid by a thermal rearrangement. A more detailed examination of these reactions revealed that crepenynic acid (I) can be rearranged to an allene (II) upon treatment with potassium t-butoxide at room temperature. This allene is thermally rearranged to a conjugated triene (III) simply by heating it on a steam bath 2 hours in 70% yield. III is comprised by the previously unknown trans,cis,trans- and trans,cis,cis-8,10,12-octadecatrienoic acids, and is accompanied by other positional isomers. III is converted almost quantitatively to a cyclic acid (IV) by heating it at 235-240° for 45 minutes.

Establishment of positions and configurations of double bonds in conjugated triene III was facilitated by partial reduction with hydrazine. Exploratory work was done on α-eleostearic acid to evaluate and develop the method. It was known from published work that the double bonds of unconjugated trienes



(e.g., linolenic acid) were reduced in a random fashion, but the course of hydrazine reduction of conjugated trienes had been little investigated. It was found that the hydrazine reduction of α-eleostearic acid proceeds initially in such a away as to preserve conjugation, so that both cis,trans- and trans,-trans-conjugated dienes are formed, but little or no unconjugated diene is formed. The trans-bond of the intermediate cis,trans-conjugated diene is reduced somewhat faster than the cis-bond. When applied to triene II from crepenynic acid, results were obtained indicating that the Δ^8 bond was trans, the Δ^{10} bond was cis, and that the Δ^{12} bond was a mixture of cis and trans.

Evidence was obtained in the course of this work that the unsaturated system of a conjugated triene migrates to some extent as a unit during GLC analyses, so that analysis of such trienes isolated by preparative GLC must be interpreted with care.

The cyclic acid (IV) obtained from crepenyate readily dehydrogenated to an aromatic acid, and also was reduced to a saturated cyclic acid. Either of these derived cyclic acids might be of interest for commercial development, e.g., for low-temperature lubricants and modifiers for alkyd resins.

2. The seed oil of Crepis vesicaria is quite different in composition from that of C. foetida. C. vesicaria seed oil has been shown to contain 48% of an epoxy acid which has been characterized as vernolic acid, the same one found in Vernonia anthelmintica seed oil. The seed oil of Euphorbia lagascae (family Euphorbiaceae - CPR I) was also found to have a high content of epoxy acid (57%); this was likewise shown to be vernolic acid. E. lagascae is the second example of a high-epoxy seed oil found in the Euphorbiaceae. Thin-layer

chromatographic studies of both these oils indicated that, in contrast to Vernonia oil, comparatively little trivernolin is present among the triglycerides. Instead, it appeared that there is more or less random distribution of vernolic acid in these triglycerides. Crepis vesicaria and Euphorbia lagascae present two interesting new possibilities for development of high-epoxy oilseed crops.

3. Echium plantagineum (CPR good) seed oil was selected as a representative of oils of the borage family. This had been shown by screening results to have an appreciable content (13.1%) of unconjugated tetraenoid acid as well as 9.7% of an unconjugated triene different from the conventional linolenic. These acids have now been characterized completely as all-cis-6,9,12,15-octadecatetraenoic acid and all-cis-6,9,12-octadecatrienoic acid. Nonconjugated C_{18}-tetraenoid acids were not known to occur in higher plants prior to their discovery in the borage family.

4. Helichrysum bracteatum (CPR good) seed oil (family Compositae) was found to contain a new hydroxyacetylenic acid as well as a variety of other unusual fatty acids. Complete composition of the oil is as follows:

	%		%
palmitic	9.0	coronaric (epoxy)	14.0
stearic	3.3	conjugated dienol	4.4
oleic	8.7	helenynolic	7.2
linoleic	35.8	unknowns	2.6
crepenynic	9.5	unsaponifiables	5.5

Thus there is a total of 38% unusual acids in Helichrysum oil. Helenynolic acid has been characterized structurally as follows:

$$CH_3-(CH_2)_4-C{\equiv}C-\overset{t}{CH}{=}CH-\underset{\underset{OH}{|}}{CH}-(CH_2)_7-CO_2H$$

This new acid is the first found in nature that has the grouping -C≡C-CH=CH-CHOH- in the absence of further conjugation. This acid differs considerably in its chemical properties from dimorphecolic acid, which has the analogous conjugated dienol grouping (-CH=CH-CH=CH-CHOH-). When refluxed with 0.1 N methanolic HCl, dimorphecolic is readily dehydrated, but helenynolic is converted to the corresponding methyl ether in 70% yield and undergoes little, if any, dehydration.

5. Calea urticaefolia (family Compositae) seed oil has been shown to contain as a major constituent (31.2%) fatty acid trans-3,cis-9,cis-12-octadecatrienoic acid. This was the second fatty acid to be discovered in the New Crops program having an isolated trans-double bond together with the familiar methylene-interrupted cis-diene system of linoleic acid. The earlier example, trans-5,cis-9,cis-12-octadecatrienoic acid, was discovered in the seed oil of Thalictrum polycarpum.

B. Characterization work in progress.

1. *Cardamine impatiens* (family Cruciferae - CPR I) seed has been found to yield an oil that is quite unusual both in constituent acids and in triglyceride structure. The mixed fatty acids of this oil contain approximately 24% of saturated vicinal dihydroxy acids, all of which have the *erythro* configuration. These dihydroxy acids have a series of chain lengths--C_{18}, C_{20}, C_{22}, and C_{24}. The nonhydroxy or "normal" acids are of the sort usually found in cruciferous oils with linoleic and erucic acids predominating.

It has been shown that the lipid obtained by petroleum ether extraction of *Cardamine impatiens* is actually a glyceride. However, the vicinal dihydroxy groupings apparently do not occur as such in these glycerides. Present lines of evidence suggest that one of each pair of is acylated in some manner, probably by another fatty acid.

It is expected that *Cardamine* oil will have potential as a liquid wax, as a lubricant, or as a source of polyfunctional chemical intermediates. A number of plant seed oils are now known which have high concentrations of monohydroxy acids, but *Cardamine* oil appears to be the first found to contain more than trace amounts of dihydroxy acids.

2. Work on the unique allenic fatty acid of *Leonotis nepetaefolia* has progressed so that stable oxidative cleavage products from both ends of the molecule can now be isolated. This was accomplished by permanganate-acetic acid oxidation. Present lines of evidence point to the following structure for the acid:

$$CH_3\text{-}(CH_2)_{10}\text{-}CH{=}C{=}CH\text{-}\underset{\underset{CH_2}{|}}{CH}\text{-}CH\text{-}CO_2H$$

This structural assignment must be regarded as tentative and subject to revision.

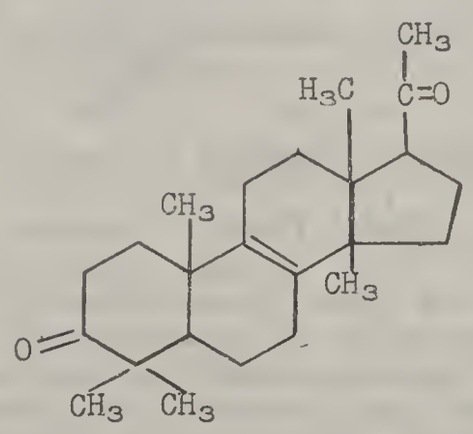
3. The seed lipids of *Briza spicata* (family Gramineae - CPR I) are quite unusual in that they contain little, if any, conventional triglycerides, but instead are comprised mainly by glycolipids. These glycolipids have been separated into a series of fractions by countercurrent distribution differing in polarity. One fraction, the predominant one on a weight basis, has been shown to be a mixture of digalactoglycerides. When subjected to mild alkaline hydrolysis, these yield digalactoglycerol and a mixture of common fatty acids--chiefly the familiar palmitic, oleic, and linoleic acids. This digalactoglycerol is probably identical with the one originally characterized by Carter and coworkers. The *Briza spicata* lipids have powerful emulsifying properties and may be potentially interesting for commercial development.

4. Acanthosyris spinescens (family Santalaceae) seed oil has been found to contain a series of acids that are conjugated enynes, some of which contain terminal bonds in addition. About 20% of the constituent acids are hydroxy acids and, surprisingly, about 34% of the Acanthosyris acids have normal C_{17}-carbon chains. It appears to be the first higher plant found that produces normal odd-numbered fatty acids in more than trace amounts (1-3%). Related acids having enyne groupings have been reported previously in the Santalaceae, but C_{17} chain apparently had not been encountered in other genera. The hydroxy acid fraction has been found to contain at least three different acids. One of these, representing about 70% of the total hydroxy acids, has been characterized structurally as 7-hydroxy-trans-10,16-heptadecadien-8-ynoic acid:

$$CH_2{=}CH{-}(CH_2)_4{-}CH{=}CH{-}C{\equiv}C{-}\underset{\underset{OH}{|}}{CH}{-}(CH_2)_5{-}CO_2H$$

Preliminary studies on the chemistry of this acid suggest that its hydroxyl group will be significantly different from both dimorphecolic and helenynolic acids in reactivity, and that it may provide new insight into the reactions of conjugated systems found in seed oils.

5. Penstemon spectabilis (family Scrophulariaceae - CPR good) seed oil contains 16% unsaponifiables which have been resolved into three major fractions by a countercurrent procedure. From one of these, intermediate in polarity, has been obtained a tetracyclic triterpenoid tentatively assigned the empirical formula $C_{24}H_{36}O_2$. On the basis of chemical, IR, NMR, and mass spectral data, the



above compound may have the above structure. This was suggested by Dr. N. S. Bhacca of Varian Associates, who has done some collaborative work on this substance.

III. Selected Species or Components That May Merit Further Study

A. Oils with usual unsaturated acids. Species producing oils rich in the common unsaturated acids are not listed in this report. Selection of any of these materials for evaluation should be based not only on the top few in chemical composition, but on a balance of composition and agronomic prospects. An excellent plant type with less than 60 percent linolenic might well be chosen in preference to a species with 70 percent linolenic but a plant form unlikely to be improved for mechanical production and harvest.

1. Tetraenes and trienes, nonconjugated. GLC shows 60 percent or more linolenic acid in 25 species analyzed since the last report. In addition, the borage family contains linolenic acid, 6,9,12-18:3, and 6,9,12,15-18:4 in varying proportions and in one instance providing the highest iodine value, 225, yet encountered in an unfractionated oil. The highest amounts of these components encountered in this report period are: *Euphorbia parryi*, 76 percent 9,12,15-18:3 (CPR I); *Symphytum officinale*, 27 percent 6,9,12-18:3; *Lithospermum tenuiflorum* and *Moltkia aurea*, 16 percent 6,9,12,15-18:4 (CPR II).

2. Dienes. Fourteen species were found in this period to produce oils containing 75-81 percent 18:2.

3. Monoenes (other than Umbelliferae and high erucic oils). Only two species were found with more than 70 percent apparent oleic acid and the CPR's of these are IV and V. *Leavenworthia torulosa* oil contained 53 percent 20:1 (CPR I).

B. Epoxy oils. Screening for epoxy oils has been most profitable in that five species were discovered to have significant amounts of epoxy acids. These are:

Sp 44776	*Euphorbia lagascae*	Euphorbiaceae	62%	CPR	I
Sp 44437	*Crepis vesicaria*	Compositae	48%		II
42942	*Crepis occidentalis*	Compositae	34%		
Is 42996	*Cephalaria joppica*	Dipsacaceae	29%		I
Sp 43090	*Cephalaria leucantha*	Dipsacaceae	27%		I

Euphorbia lagascae is the first member of the Euphorbiaceae we have found to produce vernolic acid, but Gunstone has previously reported it in a different genus (*Cephalocroton*) of the family. *Crepis vesicaria* has the highest amount we have found in the Compositae, except for *Vernonia*. *C. occidentalis* contains some 10 percent crepenynic acid in addition to the epoxy acid. The HBr reactive acid in the *Cephalaria* has not been identified. It is titratable at 3° and 55°, shows no unusual absorption in the UV, but only weak absorption in the IR. It occurs in other members of the family to perhaps 8-12 percent. No epoxy acids have been characterized in the family Dipsacaceae.

C. Oils with conjugated unsaturation.

Impatiens edgworthii	CPR II	61% tetraene	34% 18:4, 11% sat. by GLC
Calendula arvensis	CPR I	41-46% triene	
Jacaranda ovalifolia	CPR IV	27-32% triene	
Valerianopsis polystachya	CPR III	33% triene	14% sat.
Trichosanthes cucumerina	CPR III	47%	
Prunus mahaleb		33%	
Cucurbita palmeri		37%	
Maximowiczia sonorae	CPR III or IV	35%	19% sat.

The 61 percent tetraene (by UV) in oil of Impatiens edgeworthii is the highest concentration we have found. The lower figure found by GLC probably results from loss of the highly unsaturated acids in the column. If so, the percentage of saturated acids should be corrected downward. The oil appears to contain 10 percent of water-soluble acids (acetic?) not considered in the GLC calculation of composition.

D. Acetylenic acid. In addition to Crepis foetida, crepenynic acid has been found in C. foetida ssp. rhoeadifolia (36 and 54 percent) (CPR I), C. thomsonii (60 percent) (CPR I), C. rubra (33 percent), and Crepis sp (27 percent). With the two species producing vernolic acid, these are all the Crepis samples we have screened.

GLC shows 12 percent crepenynic acid in oil from Centaurea gymnocarpa (CPR I). Analysis of 17 other Centaurea species shows numerous unidentified components, but in only one species is crepenynic acid reported (4 percent). Osyris alba oil (CPR III) contains 81 percent ximenynic acid by UV and Acanthosyris spinescens 69 percent (CPR V).

E. Unusual	Presumed composition by anal.	CPR
Cuspidaria sp.	20% keto acids	IV
Chamaepeuce hispanica	12% C 24.4	I
Rapanea laetevirens	solid	IV
Chrysanthemum sp.	terminal methylene by IR	
Lannea coromandelica	25% loss in GLC	V
Pachira aquatica	approx. 60% palmitic, 25% cyclopropenoid	IV
Bignonia tweediana	50% palmitoleic	IV
Helenium microcephalum	partly solid; hydroxyl by IR	I
Xylosma longifolium	27% chaulmoogric	IV
Ephedra foliata	20% 20:3 and 20:4	IV
Dimocarpus longan	17% 19:1?	V
Myrsine africana	Hydroxy acids	IV
Teucrium (various)	5,9,12-triene in new family	II-III
Lithraea brasiliensis	hydroxyl, carbonyl	V
Anemone cylindrica	6,9,12-triene in new family	II
Euryops athanasia	30% loss in GLC	III
Hypericum androsaemum	50% loss in GLC	III
Briza spicata	solid	I
Briza maxima	solid	I

F. Miscellaneous. At the January 1962 meeting, the ash content of the hull on Onosmodium seed was reported as 50 percent with spectrographic evidence that calcium was a major constituent. Recent analysis shows the ash contains 29 percent calcium. Such an accumulation of calcium suggests the possibility that strontium might also be accumulated and that the plant might be useful in removing radioactive fallout.

IV. Methods Developed for Evaluation of Oils

Gas-Liquid Chromatography of Sterculic Acid-Containing Oils. In response to an inquiry by Dr. M. R. Mills uf Unilever Limited, Kent, Great Britain, a procedure was developed for GLC analysis of Bombax oleagineum oil. Dr. Mills had not been able to detect any sterculate peak with his chromatograph using the procedure we had recommended earlier.

We have found that at low column temperatures, such as 168° C., the liquid phase level had to be lowered to about 10%, and the sample size injected kept high. The minimum sample size was determined by running several injections and plotting the percentage of sterculate as determined by each run against the sample size. Above a certain level, the sterculate composition remained fairly constant. The column could not be "conditioned" by addition of several large doses. Return to a smaller sample size diminished the sterculate percentage as before.

Since the sample size cannot be increased substantially with β-ionization detectors, which is the type used by Dr. Mills, it was recommended that the column bath temperature be raised above normal operating temperatures. We have found that at temperatures 30°-40° C. above normal, absorption of sterculate in the column was about completely eliminated, resulting in little or no difference in sterculate composition even in widely differing sample sizes. Dual column thermal conductivity and single column flame ionization units within the same gas chromatograph gave identical results when operated under identical conditions.

Quantitative Hydrogenation of Oils by Use of Brown Hydroanalyzer. A procedure was developed to adapt the automatic-titrating hydroanalyzer to seed oils for rapid determination of unsaturated carbon-carbon bonds. Statistical methods were applied to evaluate the precision of the method. When the hydrogen-iodine value of an oil determined by the hydroanalyzer differs significantly from the iodine value by the Wijs method, the presence of unusual unsaturations such as acetylenic and conjugated double bonds is indicated. The hydroanalyzer can be used to monitor the degree of unsaturation in an oil every 2 to 5 minutes, 50 times or more by injection of 100 μl samples.

DEVELOPMENTAL WORK ON OILSEEDS

NU - Erucic Acid Oilseeds

A. Crambe Seed Analyses and 1964 Plantings

1. Present supply of crambe seed. The total amount of crambe seed available from the 1964 crop for planting and processing includes 27 tons from Oregon, 10 tons from Idaho, and 2.5 tons from Wisconsin. An additional 600 pounds has been promised (Oregon and Montana), with a few other small lots totaling perhaps a ton. On hand at Peoria are some 6 tons including 5,700 pounds of the 1963 crop received from the Farmers Cooperative Grain Company, Sidney, Nebraska.

2. Crambe composition. Over 75 samples of crambe have been analyzed, many of them in more than one form. Practically all samples are received in pods with varying amounts of free seed. Analysis of a representative sample, after removal of foreign matter, gives the composition "as received," but does not necessarily represent composition "as grown" because of the seed that have been threshed out during harvest. Separation and analysis of intact pods may not give true representation of the material "as grown" because the pods that are threshed may be bigger, more mature or differ from the bulk of the sample in some other way. Many samples have been analyzed as "seed plus pericarp," including both, some as received and some as intact pods only.

Almost all samples have also been analyzed as clean seed. Oil content of seed plus pericarp ranged from 24-40 percent with the mode at 31-32 percent. Oil content of seed only ranged from 36-54 percent with rather uniform distribution from 40-48 percent. A typical result for oil content could be considered as 45 percent. The three samples at 53-54 percent came from Montana, Oregon, and Wyoming in 1962 and 1963. Protein content of "seed plus pericarp" ranged from 18-29 percent with most samples 22-25 percent. In the seed itself, protein ranged from 22-37 percent with most samples between 27-31 percent. Calculated crude protein in the oil-free meal ranged from 46-58 percent with the mean at 51-52 percent. Erucic acid content of the oil ranged from 51-60 percent, except for two samples from Alaska that contained only 40 percent. The mode was 57 percent.

One sample was carefully hand-separated, and selected analyses of the components are tabulated here:

Crambe Fractions

	Pericarp	Seed coat	Cotyledon	Hypocotyl
Proportion	40	5	49	6
Proportion of seed	--	8	82	10
Oil, % DB	0.4	17	55	38
Crude protein, % DB	4	23	23	34
Ash, % DB	8	4	4	5
Erucic acid, % in oil	40	46	59	44
Thioglucoside, % of oil-free meal, DB	0.1	2.1	10.9	13.0
Calcium, % of oil-free meal, DB	2.2	1.0	1.0	0.7
Lysine, g./16 g. N	1.5	7.4	5.5	5.6
Methionine, g./16 g. N	0.3	1.2	1.5	1.8
Hydroxyproline g./16 g. N	1.1	5.9	0.0	0.0

Eight of 11 samples of crambe specially selected by a Texas AES agronomist contained 42-47 percent oil while all 10 introduced samples (Russia) received at the same time contained 40-42 percent oil. Oils from these same eight selections were higher in erucic acid (57.2-58.8 percent) than the introductions (55.4-57.1), although the difference is within the error of the method. The consistency of the results, however, suggests that the difference in this instance is real. Analysis of further selections should be made to see if the trend continues. In one fertilization experiment, varying amounts of nitrogen had no effect on seed or oil composition. In another study, increases in applied nitrogen resulted in increases in crude protein and decreases in oil content but had no noticable effect on erucic acid content of the oil. The explanation for the difference in response is unknown but it is suggested that the initial fertility levels might have been distinctly different.

B. Chemical Investigations on Crambe Oil

Introduction

In the preparation of derivatives designed to promote and expand the utilization of crambe seed oil, products aimed at large volume industries have received major emphasis. Considerable effort has been applied to the extension of earlier promising preliminary results in the plasticizer field, and a new and ingenious approach in this connection involves the preparation of a plasticizer that can be internally bound chemically in vinyl resins, namely, a vinyl alkyl mixed ester of brassylic acid. In an attempt to gain an additional economic advantage for crambe oil products, some of the most recent studies have employed unfractionated crambe acids and the mixed dibasic acids obtained by oxidation of them rather than purified erucic and brassylic acid, respectively.

Preparation of Brassylic and Mixed Crambe Dibasic Acids

Within the limits of the analytical methods employed, the cleavage of erucic acid by ozonization appears to be nearly quantitative, and as indicated in the previous "Progress Report on New Crops Research," the principal problem lies in purification of the brassylic acid formed. Removal of the byproduct pelargonic acid by vacuum distillation followed by crystallization of the residue remains the best method, though some superior crystallization solvents, notably toluene and xylene, have been employed instead of ethyl acetate and ethyl acetate-hexane. The highest recovery of brassylic acid yet obtained was 83%. The product was 88% brassylic acid and contained as main impurities homologous dicarboyxlic acids but only 0.4% pelargonic acid. The starting material for the ethylene oxide adducts and alkyl half-ester, described in the sections "Brassylic Acid Derivatives as Plasticizers for Vinyl Resins" and "Brassylic Acid Ethylene Oxide Adducts" which follow, was prepared by crystallization of crude (52%) brassylic acid from xylene followed by decolorization with a silicic acid-Celite column and was more than 99% pure with 70% of the brassylic acid being recovered.

By way of investigating a possible new method of purification, a synthetic mixture of the methyl esters of pelargonic and brassylic acid was separated by fractional vacuum distillation. Although this involves two additional steps (esterification and saponification), the preliminary experiment indicates that almost colorless brassylic acid completely free of pelargonic acid can be obtained with almost quantitative recovery. Incidentally, dimethyl brassylate essentially free of monobasic esters but containing small quantities of homologous diesters is now commercially available at $2 per pound from Emery Industries.

The pilot-plant scale ozonization involving decomposition of the intermediate ozonides via hot air oxidation has been investigated in the Welsbach Corporation laboratories, under financial sponsorship of the Northern Division. An emulsion composed of 1 part erucic acid, 1 part propionic acid, 2 parts water and 0.01-0.10% wetting agent was maintained at 30°-35° C. while

110-115% of the theoretical amount of ozone was added. Oxidation of the ozonides was accomplished by blowing air through while the temperature of the reaction mixture was held at 60°-70° C. for the first hour, 80°-90° C. for the second and 100°-110° C. for two additional hours. Then the mixture was cooled to 10°-15° C., and the crude product removed by filtration. Analysis of a sample sent to NU by GLC of n-butyl esters indicated the following composition:

Brassylic acid	33.5%
Pelargonic acid	18.0%
Propionic acid	41.0%
Unidentified	7.5%
Water	0.7% (by Karl Fischer titration)

Recrystallization of the crude product from "naphthol spirits" (obtained from American Mineral Spirits Co., b.p. 160°-170° C.) gave a hard white powder, which was analyzed at NU by GLC of the methyl esters and found to contain 95.4% brassylic acid and only 0.3% pelargonic acid. Arrangements have been made for Welsbach to prepare brassylic acid from 800-1,000 pounds of erucic acid by this procedure.

Attention at NU has recently turned to use of mixed acids as starting materials. The mixed dibasic acids employed in the preparation of the 2-methylpentyl esters described in the section "Brassylic Acid Derivatives as Plasticizers for Vinyl Resins--Diesters" were prepared by ozonolytic cleavage of unfractionated crambe acids followed by vacuum distillation to removed most of the monobasic acids and had the following composition:

Azelaic acid	30.4%
Undecanedioic acid	4.8%
Dodecanedioic acid	1.5%
Brassylic acid	50.7%
Pentadecanedioic acid	3.0%
Pelargonic acid	3.0%
Others	6.6%

To obtain similar material in quantity for future studies, arrangements have been made with Emery Industries for the preparation of several hundred pounds of mixed dibasic acids from crambe acids supplied by NU.

Brassylic Acid Derivatives as Plasticizers for Vinyl Resins

Diesters. The preparation at NU and testing at EU of esters that were listed as "in process" in the previous "Progress Report on New Crops Research" (January 1963) has been completed, and 4 additional products have been synthesized and tested making 21 in all. The earlier described desirable poly (vinyl chloride) plasticizer properties have generally been retained as shown in Tables I and II, but the most striking results were obtained by taking advantage of complementing favorable aspects of the n-butyl and cyclohexyl derivatives. When brassylic acid was

Properties of Molded PVC Sheets Containing 32% Brassylic Acid Diesters

Diester plasticizer	Compatability	Tensile psi	Modulus 100% psi	Elongation %	Torsional stiffness temp., °C. $T_{135,000}$	Torsional stiffness temp., °C. $T_{10,000}$	Migration % wt. loss	Volatility % wt. loss
Methyl[a]	Fair	2440	630	430	-45	-14	19.0	23.0
Ethyl	Excellent	2460	700	320	-50	-16	18.3	14.0
Propyl	Excellent	2320	710	330	-53	-16	21.1	5.8
Isopropyl	Excellent	2340	790	330	-52	-20	18.5	12.0
Butyl	Excellent	2300	740	355	-55	-18	22.1	3.2
Isobutyl	Excellent	2580	890	395	-47	-14	17.8	3.4
Hexyl	Good	2280	860	380	-51	0	22.6	1.4
2-Ethylbutyl	Good	2450	990	380	-53	-14	18.7	1.4
2-Methylpentyl	Excellent	2330	860	370	-61	-21	22.2	1.6
4-Methyl-2-pentyl[a]	Poor	2120	1160	270	-53	+ 6	19.5	8.4
Cyclohexyl	Excellent	2510	1000	370	-29	- 2	5.8	0.8
Octyl	Very poor							
2-Ethylhexyl[a]	Fair	2260	1120	320	-63	+ 1	19.7	4.1
Decyl	None							
2-Methoxyethyl	Excellent	2370	670	320	-45	-21	18.0	2.0
2-Butoxyethyl	Good	2280	880	310	-54	-16	20.5	1.7
2(2-Butoxyethoxy) ethyl	Good	2550	990	370	-46	-10	17.9	1.7
Butyl 2-ethylhexyl	Excellent	2240	880	340	-58	-16	21.0	2.9
Mixed esters: butyl and 2-ethylhexyl	Excellent	2240	890	360	-57	-16	20.8	2.0
Mixed esters: butyl and cyclohexyl	Excellent	2400	940	280	-45	- 5	16.5	2.2
2-Methylpentyl esters of mixed dibasic acid	Excellent	2280	870	380	-56	-16	20.5	4.5
Controls (Commercial):								
Di-(2-ethylhexyl) adipate		2380	890	340	-53	-17	17.6	3.4
Di-(2-ethylhexyl) sebacate		2420	970	340	-57	-12	19.1	2.5
Di-(2-ethylhexyl) azelate		2420	960	340	-53	-14	18.3	1.6
Di-(2-ethylhexyl) phthalate		2570	1150	365	-29.5	0	3.9	1.7

a/ Exudate present throughout the tests.

TABLE II

Heat and Light Stability of Molded PVC Sheets Containing 32% Brassylic Acid Diesters

Diester plasticizer	Heat stability (hours to failure at 160° C.)	Light stability Hours	Light stability Failure
Methyl	5.4	336	Hardening
Ethyl	5.4	720	Discoloration and hardening
Propyl	5.4	720	Discoloration
Isopropyl	5.0	576	Hardening
Butyl	6.0	768	Discoloration
Isopropyl	6.2	768	Discoloration
Hexyl	6.9	168	Exudation
2-Ethylbutyl	6.9	288	Exudation
2-Methylpentyl	6.7	240	Exudation
4-Methyl-2-pentyl	6.3	0	Exudation
Cyclohexyl	6.9	888	Discoloration
Octyl		Incompatible	
2-Ethylhexyl	6.9	24	Exudation
Decyl		Incompatible	
2-Methoxyethyl	5.5	600	Exudation
2-Butoxyethyl	3.8	144	Exudation
2(2-Butoxyethoxy)ethyl	3.0	120	Exudation
Butyl 2-ethylhexyl	6.4	144	Exudation
Mixed esters: butyl and 2-ethylhexyl	6.3	168	Exudation
Mixed esters: butyl cyclohexyl	6.7	528	No failure[a]
2-Methylpentyl esters of mixed dibasic acids	6.3	192	Exudation
Controls (Commercial):			
Di-(2-ethylhexyl) adipate	6.4	600	Exudation
Di-(2-ethylhexyl) sebacate	6.8	240	Exudation
Di-(2-ethylhexyl) azelate	7.0	312	Exudation
Di-(2-ethylhexyl) phthalate	6.9	768	Discoloration and spotting

a/ Test still in progress.

esterified with a mixture of n-butanol and cyclohexanol, the product possessed excellent low-temperature plasticizer properties approaching those of n-butyl brassylate while at the same time maintaining the better heat stability of cyclohexyl brassylate. In general the mixed brassylate proved to be a much better low temperature plasticizer than dioctyl phthalate and to offer several advantages over dioctyl sebacate, such as heat stability and stability from exudation due to light with a slightly lower migration loss.

Finally, the quite acceptable plasticizer properties of the 2-methylpentyl esters of mixed crambe dibasic acids are encouraging in that they suggest an avenue for future investigation that might lead to a product with a considerable economic advantage. The commercial potential of an improved plasticizer for vinyl resins is indicated by the fact that more than a hundred inquiries have been received from industrial organizations regarding esters of brassylic acid.

Vinyl Alkyl Mixed Esters. Brassylic acid mono-ester of 2-methylpentanol, the intermediate to vinyl 2-methylpentyl brassylate, was prepared by p-toluenesulfonic acid-catalyzed esterification of the free acid and alcohol in refluxing toluene. The mono-ester constituted 50% of the products, as predicted, and was recovered by fractional crystallization in fair yield, 60% at 98% purity. Further purification to 99.8-100.0% was achieved by silicic acid column adsorption of the contaminant with quantitative recovery of the mono-ester. In an effort to improve the yield by use of a re-cycling process, the mother liquor and unreacted brassylic acid from the first reaction were mixed with fresh brassylic acid and 2-methylpentanol, and re-subjected to the esterification conditions. The recovery of purified mono-ester in this second reaction was somewhat lower due to the accumulation of colored products that tended to inhibit its crystallization.

It is anticipated that the vinylation of the mono-ester can be effected either by acetylene addition or by ester interchange with vinyl acetate. The resulting vinyl 2-methylpentyl brassylate may then be copolymerized with vinyl chloride. This fatty ester internal plasticizer will contain an added ester linkage and a side chain which may manifest the same desirable plasticizing properties of 2-methylpentyl brassylate diester without the loss by volatilization or exudation. Extreme care has been exercised to remove brassylic acid from the mono-ester product to avoid subsequent formation of divinyl brassylate, which has been found earlier to be a strong crosslinking agent that forms highly insoluble polyvinylchloride copolymers.

Brassylic Acid Ethylene Oxide Adducts

Potassium hydroxide has been found preferable to BF_3 as a catalyst for the reaction of ethylene oxide with brassylic acid. The reaction readily takes place at 125°-150° C. in the absence of solvent when the oxide is added directly to the acid containing 1% of the catalyst. In this manner 11 products ranging from 1 to 100 moles of ethylene oxide per mole of brassylic acid were prepared. Some were deionized in alumina columns or washed with boiling aqueous saturated NaCl to give a total of 22 products. Average molecular weight of each product was determined by both chemical determination of hydroxyl and saponification values and by osmometric means. These adducts cover the whole range of oil-in-water to water-in-oil surfactant range. Surface tension measurements show these to be comparable to commercial detergents which have similar hydrophile-lipophile balance.

Erucic and Crambe Acid Amides

Following the work of Mod, Magne and Skau of SU on long chain fatty amides as plasticizers for vinyl resins, piperidides and morpholides of erucic and crambe acids were prepared at NU and evaluated at SU. Although all four amide plasticizers were compatible with the resin at the 35% level, the piperidides exhibited better compatibility than the corresponding morpholides. (Table III)

TABLE III

Fatty Amide Plasticizers-Vinylite VYDR Compositions (35% Plasticizers)

Plasticizer	Tensile strength, psi	100% Modulus psi	Elongation %	Torsional stiffness temp., ° C. $T_{135,000}$	$T_{10,000}$	Volatility loss, %
N-erucoylpiperidine	2930	1460	380	-39.0	-11.0	0.34
N-erucoylmorpholine	3110	1620	350	-28.5	- 3.5	0.04
Piperidide of crambe acids	2750	1270	360	-32	- 8.5	0.68
Morpholide of crambe acids	2970	1430	360	-24	- 0.5	0.38
Di-(2-ethylhexyl) phthalate (control)	3050	1610	330	-23.5	- 3.0	1.50

The amide plasticizers exhibit better low-temperature flexibility with less volatility loss than the dioctyl phthalate control. The piperidides show better low-temperature flexibility than the morpholides; both have exceptionally low volatility loss. The performance of the mixed crambe acid amides is significant in view of the potential low cost of the acids.

In view of the favorable results obtained, 16 additional amides are in process of preparation. Erucic and crambe acids will be reacted with the following amines: 3-methylpiperidine, 4-methylpiperidine, 4-propylpiperidine, bis(2-methoxyethyl)amine, bis(2-ethoxyethyl)amine, N-methylfurfurylamine, hexamethylenimine and pyrrolidine.

Interesterification of Crambe Oil

Directed interesterification of crambe oil offers a theoretically simple approach to obtaining trierucin of relatively high purity. Crude crambe oil contains essentially no trierucin presumably because erucic acid is incorporated biosynthetically exclusively in the α,α' positions. Interesterification at 14° C. using sodium methoxide as catalyst led to products which were fractionated at -25° C. to yield an enriched erucic fraction. It comprised half of the oil, 65% of all the erucic acid, and was 78% pure. Calculations show the oil to have formed 18% trierucin. The byproduct from fractionation may be salable as a glyceride oil rather than mixed fatty acids.

Research on Crambe by NU's Oilseed Crops Laboratory

Erucic Acid Cleavage Products. Because of the excellent plasticity and unusual heat stability imparted to poly(vinyl chloride) by certain ester-acetal derivatives of azelaaldehydic acid, analogous compounds were prepared from brassylaldehydic acid for testing in poly(vinyl chloride). Ozonization of methyl erucate in methanol and reduction of the ozonolysis products by zinc and acetic acid gave an 85% yield of methyl brassylaldehydate. Conversion of the latter to the dimethyl acetal followed by selective alcoholysis of this ester-acetal gave 2-ethylhexyl 13,13-dimethoxytridecanoate. Cleavage of the acetal group gave 2-ethylhexyl 13-methoxy-12-tridecenoate. The higher boiling points of the brassylaldehydate derivatives compared to the azelaaldehydates should remedy the relatively high volatility losses which occurred with the azelaaldehydate derivatives in poly(vinyl chloride). The samples have been sent to EU for evaluation.

Flavor and Oxidative Stability of Crambe and Some Related Linolenic Acid-Containing Oils. Because crambe, mustard seed, and rapeseed oils like soybean oil, contain linolenic acid, they have been examined and compared with soybean oil for flavor stability after accelerated storage and after exposure to fluorescent light. Tests showed that the Cruciferae oils did have similar flavor characteristics and that the addition of citric acid did improve their stability. However, when exposed to light the citric acid treated Cruciferae oils differed from soybean oil. They developed a rubbery flavor whereas soybean oil developed a grassy flavor. Oxidative stability determined by the active oxygen method confirmed results of the oven storage tests. This study tends to confirm the belief that if linolenic acid is present in an edible oil, it is the precursor to the typical off-flavor development.

C. Crambe Meal Studies

Introduction and Summary

A demand for crambe seed oil has been created, but the economics of the situation assuredly will require a good market for the coproduct meal, with use as a feed ingredient the most obvious outlet. The economical production of a nutritious and palatable meal, free from undesirable side effects, is important to the future status of crambe as a cash crop in the United States.

To achieve these objectives, the work of the Industrial Crops Laboratory has been concerned with (1) the composition of crambe meal, (2) the enzymatic transformation of thioglucosides, and (3) the removal or neutralization of those constituents or transformation products which might otherwise severely restrict the use of crambe meal as a feed ingredient. Futhermore, an understanding of compositional variations and of the course of the enzymatic transformations is essential in order to define and control processing conditions.

The limitations on the use of unprocessed crambe meal derive from both factors of unpalatability and toxicity. In earlier lamb-feeding tests at the Illinois State AES and in current cattle-feeding experiments under a cooperative agreement between NU and the Nebraska State AES, it was apparent that for ruminants at least the first factor was present. In other feeding tests with nonruminants, both factors were revealed. Recent feeding studies with processed meals indicate that the effect of these factors can be minimized by proper treatments.

The chief thioglucoside of crambe seed was isolated in pure form and identified as an optical isomer of that present in rapeseed and other members of the species Brassica. To indicate this relationship it is designated epi-progoitrin. Two of its enzymatic conversion products were separated and characterized, namely the oxazolidinethione, (R)-goitrin, and 1-cyano-2-hydroxy-3-butene. By analogy with (S)-goitrin from rapeseed, it may be anticipated that consumption of the oxazolidinethione from crambe would produce enlarged thyroids. In addition, the cyano compound contains two functional groups, hydroxyl and olefinic, which have been reported to confer increased toxicity on organic nitriles. It was found that by proper choice of conditions for the enzymatic conversion of crambe meal, the formation of (R)-goitrin could be almost completely suppressed and other conversion products, including the cyano compound, could be removed by solvent extraction.

It follows that the composition of crambe meal is profoundly influenced by prior treatment. Temperature, moisture content and pH all may influence the nature of the enzymatic conversion products which must ultimately be removed to produce an acceptable meal.

In addition to thioglucosides and their enzymatic conversion products, other bitter substances also are known to be present in crambe meal. One of these was isolated and characterized as sinapine, the choline ester of a substituted cinnamic acid. It is also eliminated from the meal by solvent extraction.

While most samples of crambe seed contain 8 to 10% of total thioglucosides, screening revealed two accessions which contained only half this amount. The obvious implication is that plant breeding experiments may lead to a variety which is inherently low in thioglucosides.

Crambe seed enjoys a favorable position with respect to the stability of its oil. Either intact or broken seeds may be kept in storage as long as 3 years without any significant amount of lipolytic change.

In summary, crude crambe seed meal contains substances which make it a poor feed ingredient. These undergo complex changes which may vary with the treatment. However, basic studies now permit assay for certain constituents, suggest criteria for improving the control of processing, provide methods for removal of the unwanted substances, and give promise of a practical process for a good and nutritious feed.

Laboratory Studies on Crambe Meal Composition

Analytical methods. Analyses of hot water extracts of meal samples for sulfate ion in the presence of added mustard myrosinase gave a measure of total thioglucosides. Similar analyses on extracts from enzyme-inactivated meals with no added myrosinase measured only the thioglucosides already converted. Nitriles and other products containing C≡N groups which may have formed from hydrolysis of the thioglucosides were quantitatively estimated by measurement of absorption of C≡N in the infrared. Sinapine and related products were estimated by a modification of the colormetric assay method of Tsagaloff. (R)-Goitrin was measured in the ultraviolet by a modification of the procedure of Ettlinger and Dateo. Qualitative thin-layer and paper chromatographic methods were developed for products formed from thioglucoside hydrolysis.

Major thioglucosides and related compounds. Of the total thioglucosides in crambe seed, 75 to 90% consists of a single substance which was isolated and its constitution established by identification of the hydrolysis products. Mustard myrosinase hydrolysis of the isolated thioglucoside at neutral pH gave a substance identified as (R)-5-vinyloxazolidine-2-thione ((R)-goitrin), an enantiomer of the (S)-goitrin derived from the thioglucoside of rapeseed. Enzymatic hydrolysis at acid pH gave both (R)-goitrin and a compound whose structure was shown to be 1-cyano-2-hydroxy-3-butene (CH_2=CH-CHOH-CH_2CN). Mineral acid hydrolysis gave pentadienoic acid (CH_2=CH-CH=CH-COOH). Thus the carbon chain and also the configuration at the hydroxyl carbon were established and the major thioglucoside of crambe is (R)-2-hydroxy-3-butenylglucosinolate, or epi-progoitrin by analogy with the progoitrin of rapeseed.

If the thioglucosides of crambe meal are enzymatically converted so as to yield no (R)-goitrin, the conversion products formed under these conditions are nevertheless extractable with aqueous-acetone, and can be isolated as an ether-soluble fraction. That this fraction is in fact derived from crambe thioglucoside was established by comparison with a similar extract from a defatted meal in which little enzymatic hydrolysis had taken place. In this case only a small amount of ether-soluble product was obtained. The aglycones isolated from the ether-soluble fraction from converted meal ranged in weight from 1.1 to 1.6% of the defatted seed. Analysis: sulfur, 15-18%; nitrogen, 9.5-12%; C≡N calculated as 1-cyano-2-hydroxy-3-butene, 65-75%. When first isolated the ether-soluble mixture was very soluble in acetone and alcohol. On standing at room temperature the liquid becomes more viscous and eventually solidifies. Under nitrogen in the refrigerator some samples have remained unchanged for over a month. 1-Cyano-2-hydroxy-3-butene has been separated and identified as part of this mixture.

Crambin. Crambe seed contains a water-insoluble polypeptide named "crambin" estimated to be present as 0.5 to 0.9% of the defatted seed meal. This crystalline substance has been shown by physical methods and by amino acid analysis to be a single component with a molecular weight of 5,000 ± 300. Performic acid oxidation of the three disulfide links in the molecule gave a water-soluble product of essentially the same molecular weight. From this result it was concluded that "crambin" is a single-chain molecule with intramolecular disulfide crosslinks.

Pigments. A bitter substance present in many of the Cruciferae is sinapine, the choline ester of 3,5-dimethoxy-4-hydroxy cinnamic acid. Contrary to earlier results, it is now possible to isolate sinapine from crambe extracts. Sinapine was obtained as the crystalline thiocyanate salt from the water-soluble portion of aqueous-acetone extracts of crambe meal. Its identity with sinapine from rutabaga was established by physical and chemical methods and by hydrolysis to sinapic acid and choline.

By the modified Tsagaloff assay for sinapine, the optical absorption was measured at 400 mμ after development of the yellow color in alkaline solution. The procedure is sensitive to less than 1 microgram of sinapine per ml. of methanol solution and the assay is reproducible to 0.02%. It was observed that conversion (hydrolysis) of crambe meals with native enzymes in the presence of limited amounts of water had no effect on sinapine content. Hence, no sinapine esterases were present in the mature seed. Extraction of the converted meals with aqueous-acetone removed essentially all of the sinapine from the meal whereas anhydrous acetone did not.

Although sinapine is the major fluorescing constituent in crambe meal extracts, other fluorescing substances also are present. Four of these have been separated from sinapine-containing extracts by preparative chromatography. At least three do not contain the sinapoyl group as shown by comparison of their ultraviolet absorption spectra and of the bathochromic shifts in absorption observed when their solutions are made alkaline. In addition, two other fluorescing substances have been separated from crambe extracts which were free of sinapine or related compounds.

Screening of crambe accessions. Analysis of 64 crambe seed accessions from 12 states including accessions from Russian, Polish, and Swedish seed sources for total thioglucosides gave a range from 7.8 to 10.3% of the air-dry defatted seed meal--except for two accessions from Montana. These two accessions contained 4.0 and 5.2% thioglucoside; they are of interest as seed sources for propagation of a variety lower in total thioglucoside content. Estimations of epi-progoitrin as a percent of total thioglucosides on ten of the accessions were all greater than 70%. Sinapine assays of some representative crambe accessions are presented in Table I.

TABLE I

Sinapine Content of Representative Crambe Meals

Accession number	Sinapine as thiocyanate salt, % of defatted meal
71078	0.52
70480	0.52
50025	0.34
17184-60-2-6	0.31
50083	0.50
50024	0.46
71057	0.27

Enzymatic Studies

Mustard myrosinase. Isolated epi-progoitrin hydrolyzed with myrosinase from white mustard seed gave quantitative sulfate ion formation which showed complete hydrolysis from pH 3 to about pH 11. From pH 2.8 to 6 a mixture of (R)-goitrin, 1-cyano-2-hydroxy-3-butene and free sulfur were the identified products of which the first two were quantitatively estimated. At pH 7 and above only (R)-goitrin was found (equivalent to more than 90% of the epi-progoitrin taken). The results are shown in Figure I. These studies provided degradation products needed for the development of analytical methods and for the further chemical studies described in this report.

Crambe myrosinase. During the early attempts to prepare crambe meals for feeding trial, it became apparent that (R)-goitrin was not always formed. This particular result was unexpected, but not without precedent as was pointed out in the previous New Crops report (January 1963, p. 21). A study of the end products of thioglucoside hydrolysis in defatted crambe meal was undertaken to determine how changes in conditions might affect the course of the conversion reaction.

The amount and temperature of water used in hydrolyzing thioglucosides in crambe meal is critical, as shown in Figure II. Both increase in volume of water and increase in temperature favor the formation of (R)-goitrin.

The product vs. pH profile for endogenous crambe myrosinase in the presence of a limited amount of water at room temperature is shown in Figure III and differs from that of mustard myrosinase (Figure I). The chief products formed at pH 5 are nitrile and related products--the same as those isolated from the aqueous-acetone extracts discussed above. At high pH, however, the principal product is (R)-goitrin. The difference between the total measured aglycones and the thioglucoside as measured by sulfate is partially due to volatile products not measured. The decline in the sum of measured aglycones at lower pH values indicates the formation of unknown product(s).

These changes in reaction products by modification of pH, temperature, and water volume admit the possibility of two enzymes being present in the myrosinase system which compete with the substrate--or an intermediate--to form divergent products.

The various seed parts of crambe differ in their content of myrosinase. The enzyme activity is highest in hypocotyl as shown in Table II.

TABLE II

Myrosinase Activity of Defatted Seed Parts

Seed part (defatted)	Enzyme activity,* μ eq./min./mg.
Pericarp	zero
Seed coat	zero
Cotyledon	0.14
Hypocotyl	0.24

*Measured as release of HSO_4^- from crude sinigrin.

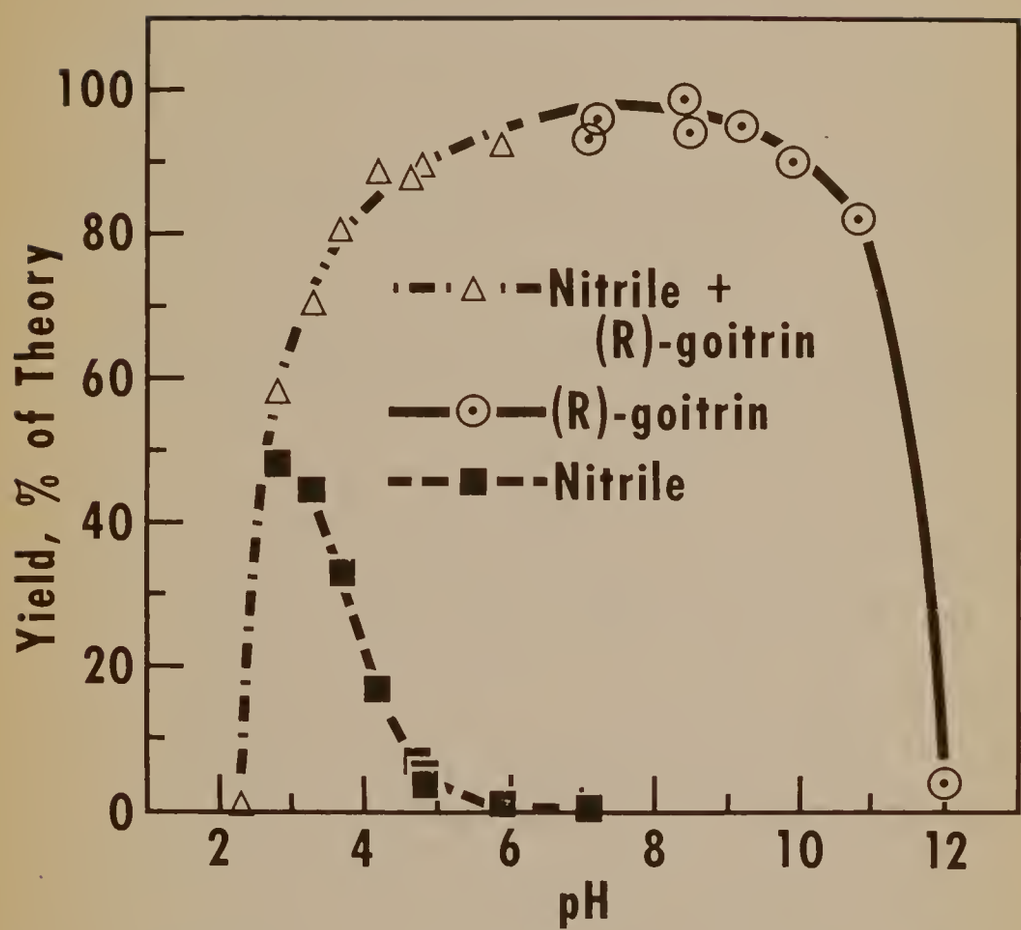


Fig. 1 Products of thioglucoside conversion as related to pH. Mustard myrosinase action on isolated *epi*-progoitrin (room temperature).

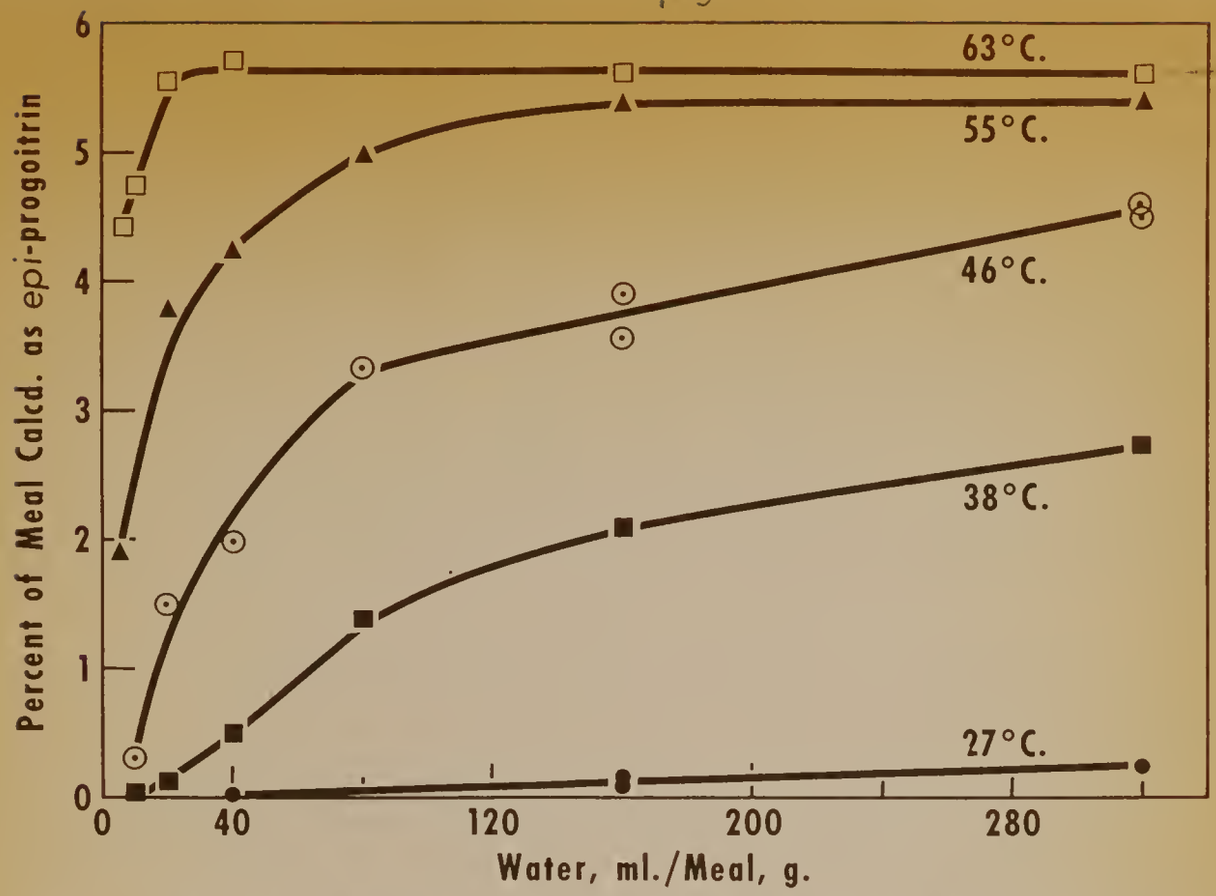


Fig. 2 (R)goitrin formation as related to dilution of seed meal at different temperatures. Total *epi*-progoitrin in meal by assay 7.0%.

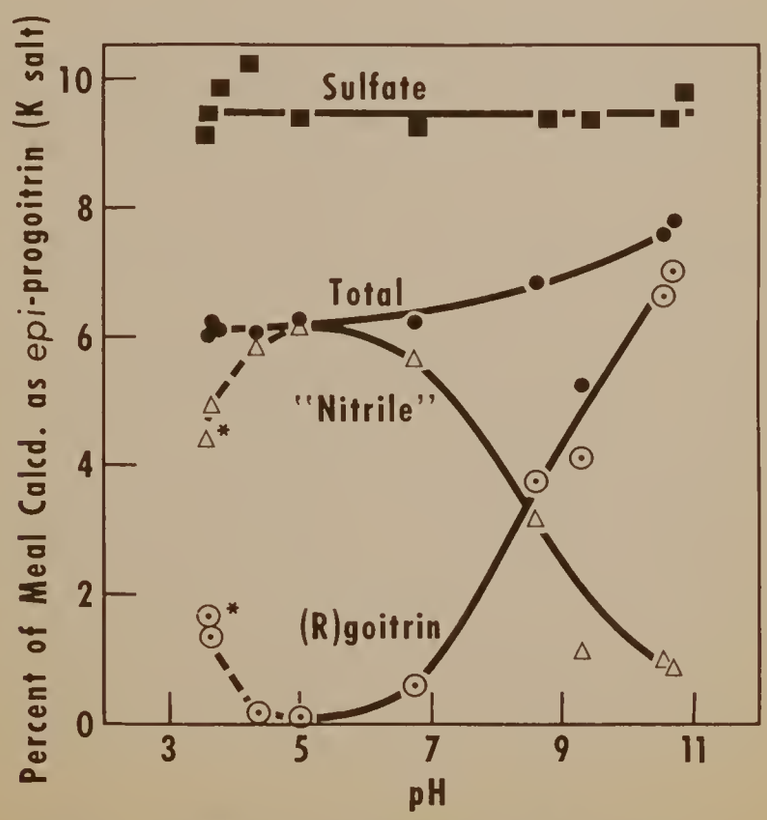


Fig. 3 Products of thioglucoside hydrolysis as related to pH. Defatted crambe meal incubated 60 min. at room temperature with 5 ml. H_2O per g. meal.

In general the thioglucosides in crambe meal appear quite stable except in the presence of moisture and myrosinase. With air-dry defatted crambe seed as test material, no thioglucosides were destroyed by dry heat for 4 hours at 113° C.; about 50% were destroyed when heated 4 hours at 128° C.; and complete destruction was observed in 12 hours at 150° C. However, the myrosinase was not completely destroyed on dry heating 4 hours at 128° C. Wet autoclaving 20 minutes at 105° C. caused partial nonenzymatic destruction; 50 minutes autoclaving at 113° C. caused almost complete destruction. Boiling carbon tetrachloride extraction of the oil from the seed caused no destruction of thioglucosides or accompanying myrosinase. Flaked seed with the oil present lost no thioglucosides on standing over 2 weeks in laboratory air. Defatted seed meal on standing 2 weeks at 100% relative humidity lost nearly all the thioglucoside due to enzymatic hydrolysis.

Crambe myrosinase is thermally inactivated by 5 minutes' exposure (in solution) at 75° C. (Figure IV). Both release of HSO_4^- and glucose are catalyzed by a water-insoluble enzyme fraction. The release of acid sulfate ion always occurs during the hydrolysis of thioglucosides, and has been used as a convenient measure of enzyme activity. The enzyme system has a broad pH optimum from pH 6 to 9 against purified crambe thioglucosides.

Crambe lipase. The stability of crambe oil to lipolysis in flaked seed at various moisture levels was previously reported. The stability of crambe oil in stored seed is now known. The seed lipase catalyzes a nearly random cleavage of triglycerides.

Oil in intact seeds or "splits" and broken seeds may be stored for long periods without accumulation of free acids in the oil. Oil from seed stored 3 years in an unheated barn was less than 2% hydrolyzed (Table III).

TABLE III

Hydrolytic Stability of Crambe Oil Stored in Intact Seed

Lot	Storage condition			Percent hydrolysis of oil
	Temp.	Time, months	Moisture, %	
I, Seed + pericarp	4-5C	36	6.1	1.6
	Unheated barn	36	4.8	1.7
II, Seed + pericarp	4-5C	36	6.4	1.3
	Unheated barn	36	5.0	1.4
III, "Splits"	4-5C	25	5.4	1.2
	Unheated barn	25	3.9	1.2
IV, Seed + pericarp	23-27C	Zero	3.7	0.5
	23-27C	12	3.7	1.2
V Cracked seed	23-27C	Zero	-	0.4
	23-27C	12	-	1.2

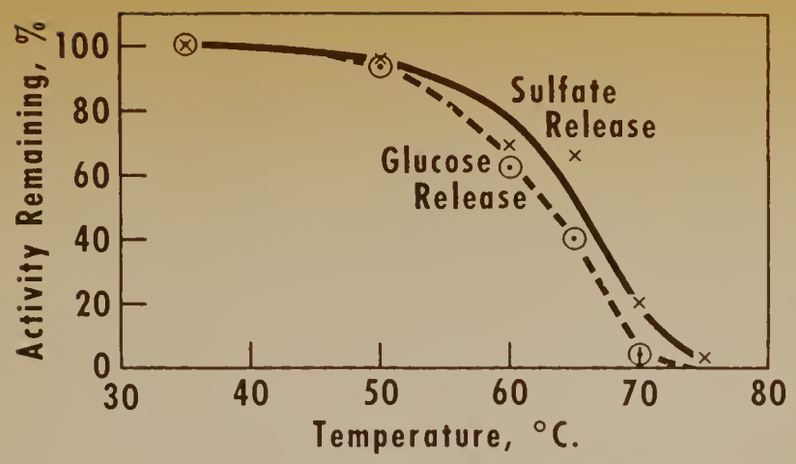


Fig. 4 Thermal inactivation of an insoluble crambe myrosinase after 5 minutes heating.

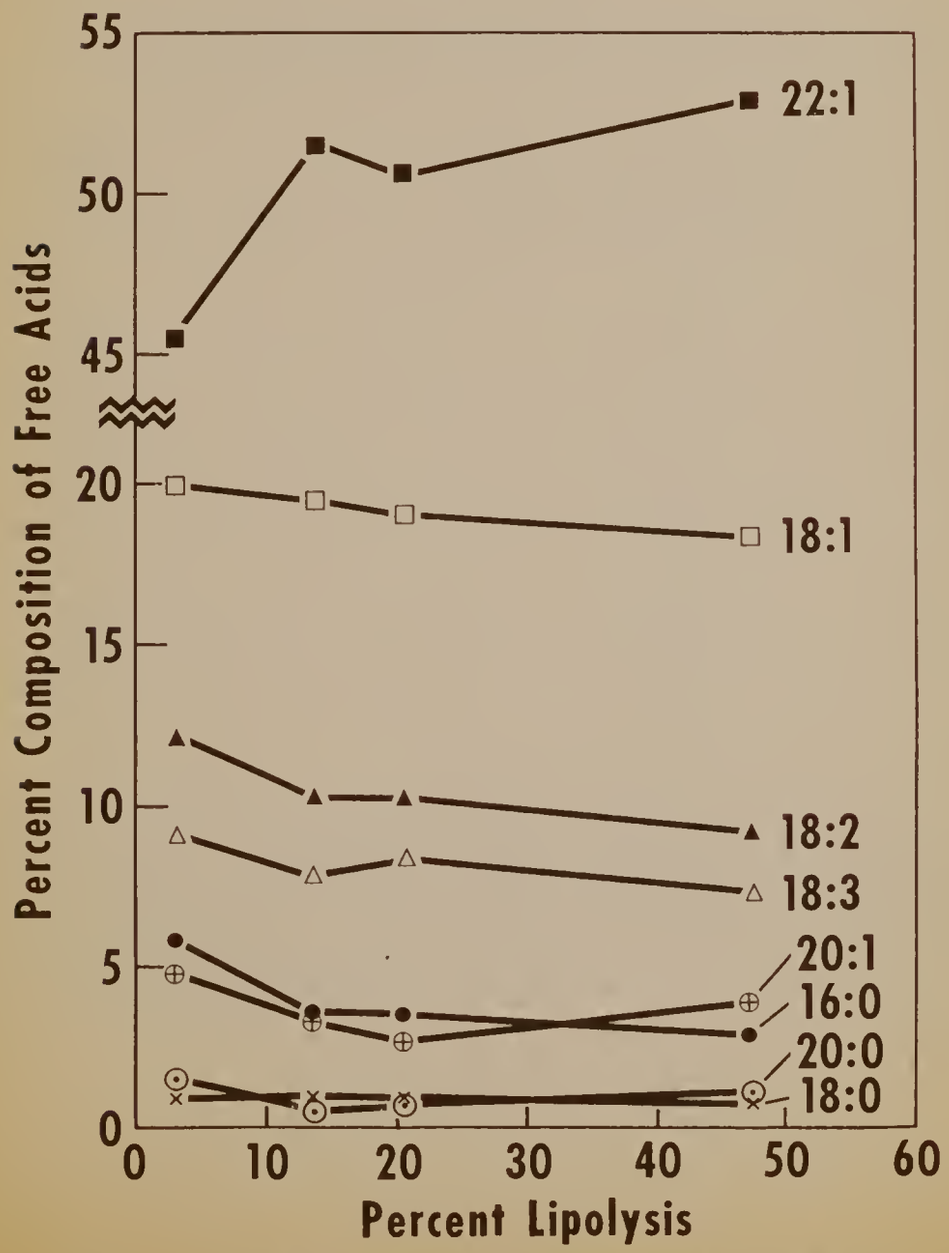


Fig. 5 Composition of free acids released by crambe lipase.

Several types of positional specificity of lipases are possible: (a) cleavage of the triglycerides may be random as in castor beans; (b) the 2-position may be preferentially split as has been suggested for Vernonia; or (c) the 1- and 3-positions may be preferentially hydrolyzed as shown in pancreatic lipase. The dormant seed lipase of crambe catalyzes a nearly random hydrolysis.

Flaked crambe seed was slurried with water and allowed to incubate at room temperature. At various time intervals (0 to 12 hours) oil was extracted from portions of the slurry; the free acids were separated by anion-exchange and chromatographed as the methyl esters on GLC. The liberated acids rapidly approach the composition of the whole oil (Figure V). The composition of the completely saponified oil is shown in Table IV. The pattern suggests a small

TABLE IV

GLC Analysis of Fatty Acid Methyl Esters Prepared from Completely Saponified Crambe Oils

Fatty acid	Percent composition as methyl esters
<16	1.2
16:0	2.5
16:1	0.3
18:0	0.6
18:1	17.4
18:2	8.9
18:3	6.9
20:0	0.7
20:1	3.5
22:0	0.3
22:1	55.4
22:2	1.2
24:0	0.6
24:1	0.5

preference for shorter-chain acids (C_{16} and C_{18}), these appearing in relatively greater abundance early in lipolysis. This abundance is not a specificity for the 2-position. In such a case $C_{16:0}$ which is esterified in the 1,3-position (Matson, F. H. and Volpenhein, R. A., J. Biol. Chem. 236, 1891 (1961)), should not be relatively abundant early in lipolysis.

Feeding and Related Studies

Unprocessed crambe. Crambe meal as judged by its high protein content and favorable amino acid balance should provide a nutritious feed supplement for livestock. To exploit these characteristics a number of toxic or unpalatable products must be removed. Although epi-progoitrin and its hydrolysis products are implicated, other bitter substances such as sinapine may also be involved.

The magnitude of the problem is indicated by the toxic nature of whole crambe seed (without pericarp) and of hexane-defatted crambe meal. These materials were fed up to 189 days to rats as shown in Table V.

TABLE V

Rat Growth Obtained with Crambe Seed Meal

Material fed	Mean wt. gain in 189 days	Material fed	Mean wt. gain in 189 days
	g.		g.
Addis control diet	262	Addis + 5% defatted crambe	238
Addis control + 30% soybean	313	Addis + 15% defatted crambe	All died in 90 days
Addis + 5% crambe seed	254	Addis + 25% defatted crambe	All died in 21 days
Addis + 15% crambe seed	134	Addis + 30% crambe pericarp	203[a]
Addis + 25% crambe seed	All died in 42 days		

[a] Discontinued after 119 days, at which time Addis control weight = 248 g.

Examination of the body organs for abnormal pathology of those animals fed the 5 and 15% levels showed enlarged thyroids diagnosed as parenchymatous goiter. The thyroids were most enlarged on those animals fed crambe seed at the higher levels. At the higher levels (15% whole seed and 5% defatted seed) enlarged, abnormal livers (and in some cases enlarged abnormal pancreases) were also observed. When whole seed meal was fed to chicks at the 15% level, the results tended to parallel those obtained with rats.

Meal conversion and extraction. Under appropriate conditions the thioglucosides of crambe may be converted, either before or after removal of the seed oil, without the formation of (R)-goitrin. The organic aglycones obtained (assayed as C≡N) can be removed from the meal by extraction with solvents containing acetone and small amounts of water. Meals have been prepared which are nearly free of thioglucoside, goitrin, C≡N, and sinapine.

Meal A. Dehulled, flaked crambe seed was moistened to 38% water content and the thioglucosides allowed to convert at room temperature. Following conversion, the paste was extracted with 2% water in acetone, then with anhydrous acetone. The aqueous-acetone removes the oil (which contains 10% free acids due to lipase activity), the thioglucoside products, and sinapine in a single step. The meal is of good quality and contains no measurable (R)-goitrin or nitrile (Table VI). Sulfur balance studies do indicate that approximately one-third of the thioglucoside aglycone may still be present in the meal.

TABLE VI

Composition of Extracted Crambe Seed Meals

	Typical defatted meal	Converted meals**				
		A	B	C	D	E
Estimated weight recovery, %*	-	88	99	97	91	90
Crude protein, d.b. %	46	55	44	46	45	56
Sulfur, d.b. %	2.0	1.86	1.84	1.72	1.43	1.76
Ash, d.b. %	8.0	-	8.0	8.2	8.5	8.0
Thioglucosides, d.b. %	9.5	0.0	0.0	0.0	0.0	0.0
(R)-Goitrin, %	0.0	0.0	0.0	0.0	0.0	0.0
Nitrile and related products, %	0.0	0.1	1.4	1.1	0.0	0.0
Pigments as sinapine thiocyanate, %	0.45	-	0.31	0.42	0.06	-
Myrosinase(s) present	+	+	+	+	+	+
Percent of total nitrogen as common amino acids and amide nitrogen	87	-	-	-	97	-
Lysine g./16 g. N	5.2	-	-	-	6.2	-
Methionine g./16 g. N	1.6	-	-	-	2.0	-
Cystine g./16 g. N	2.6	-	-	-	2.1	-

* Of defatted meal or estimated equivalent of meal in full fat seed taken for the preparation.

**See text for a description of the meals.

Feeding studies using rats indicate that meal A is acceptable (see Table VII.)

TABLE VII

Rat Growth Obtained with Converted Crambe Seed Meals

Group	Meal fed*	Duration of test	Mean wt. gain
Control for Group 1	Addis Control Diet	49 days	181 g.
1	Meal A, pair fed	49 days	165 g.
Control for Groups 2-3	Addis Control Diet	28 days	168 g.
2	Meal B, ad lib	Dead in 11 days	
3	Meal C, ad lib	Dead in 13 days	

*At 28% of the diet.

Statistically there was no difference between Meal A and its control. The pancreases were slightly larger than from the controls. Thyroid and liver weights were essentially normal.

Meal B. Hexane-defatted meal was converted by the addition of 1-1/2 ml. water per gram of meal. The product was dried and ground. This meal serves as an unextracted control. Table VII shows the toxic nature of the meal. Analyses (Table VI) indicate that both nitrile and sinapine are still present.

Meal C. Hexane-defatted meal was converted as in Meal B, after which the meal was dried and ground. Extraction with anhydrous acetone did not lessen its toxicity (Table VII) or remove appreciable amounts of the noxious nitrile or sinapine (Table VI).

Meals D and E. Meals D and E of Table VI were prepared from defatted meals using a modification of the aqueous-acetone solvent and a mixed solvent of water-acetone-hexane. The analytical data are similar to the acceptable meal A, but feeding tests are incomplete.

These meals were all high in ash because the solvents for extraction did not remove potassium sulfate formed from hydrolysis of the thioglucosides. The high inorganic salt content of the diet may have some effect on animal growth at the high levels of crambe meal fed.

Biological testing of thioglucoside and its hydrolysis products. A sample of 2,4-pentadienoic acid from acid hydrolysis of epi-progoitrin was not active as an insect attractant (Entomology Research Division). Preliminary tests of the nitrile and related products indicate some toxicity to houseflies but not at practically valuable levels.

Epi-progoitrin was markedly antibacterial against Erwinia amylovora, cause of fire blight in plants, at 1,000 and 100 p.p.m. and moderately so at 10 and 1 p.p.m. It was slightly inhibitory against Xanthomonas pruni only at 10,000 p.p.m. Tests with (R)-goitrin were inconclusive. An 80% acetone extract of defatted meal was inactive in similar testing done by Crops Research Division. No conclusions can be made as to the practical potential of these materials based on these limited tests.

D. NU Engineering and Development Process Studies on Crambe and Mustard Seed

Introduction

During the past 2 years, the Engineering and Development Laboratory has carried out research toward converting crambe meal from its inherent growth inhibitory characteristics which are quite similar to rapeseed to a meal that can be effectively used by the animal feed industry. Although the basic amino acid composition of crambe is well balanced with lysine and other essential amino acids, crambe contains thioglucosides and other antigrowth factors which seriously diminish the feed value with respect to animal growth and palatability. Several processes have been developed and others are continuing which have improved the feed value of crambe meal very markedly to give a nutritive feed supplement, however, final acceptance of these processes will depend on economics of the process as well as the type of animal species to be fed. Some of the preliminary work has indicated, for example, that the ruminant animal may be able to utilize crambe meal efficiently without extensive processing of the meal if good palatability can be achieved.

Our work during this period has encompassed many phases of the overall problem. Several basic approaches such as direct dry heat, wet heat, enzymatic conversion, toasting, solvent extraction, and combinations of these have been evaluated. Meals produced by these processes have been fed to both ruminants and nonruminant animals to give significantly improved feeding values. Considerable work has also been carried out on the analysis and characterization of the crambe thioglucosides in order to follow their behavior in processing on a qualitative as well as a quantitative basis.

In February 1964, a commercial-scale plant run was carried out successfully with the Pacific Vegetable Oil Corporation by NU purchase order. Further details about this run as well as general information on ED research carried out during the past 2 years is described below under various appropriate headings. The first two sections are carried over from our mustard seed research program reporting new research work on the essential oil composition of mustard seed and methods of following heat damage to mustard proteins during processing.

Mustard Essential Oil Composition

A gas-liquid chromatography method developed for analysis of mustard volatiles showed the essential oil to be a mixture of allyl isothiocyanate and allyl thiocyanate with traces of carbon disulfide. The composition was identical to that of mustard oils produced synthetically. Also demonstrated in this study was a heat driven equilibrium reaction between the two major constituents leading to a mixture containing approximately 92% allyl isothiocyanate and 8% allyl thiocyanate.

Simple Methods for Following Heat Damage to Mustard Protein Meals

Processed mustard seed contains a considerably higher content of reducing sugar than soybeans and other oilseeds. Consequently the browning reaction, which degrades protein, presents a greater problem in processing of the oilseeds. Several indirect methods were developed for following the effects of heat treatment on protein quality. Nitrogen solubility index, optical density of aqueous extracts, and reducing sugar content correlated well with degradation of heat-labile amino acids, such as lysine, arginine, and histidine, and gave an index of protein quality.

Analysis and Characterization of Thioglucosides

The sulfate released from the enzymatic conversion of crambe thioglucosides was determined by precipitation with barium chloride to estimate total thioglucoside content. All crambe meals analyzed were in the range of 10-12% thioglucoside content on a fat-free, moisture-free basis. A volumetric procedure based on the reaction of silver nitrate with thioglucosides confirmed these results. The thioglucoside sulfur calculated from these analyses when added to the protein sulfur was found to give a good balance with total sulfur.

Approximately 5% of the total thioglucosides converted to volatile isothiocyanates upon enzymatic hydrolysis while the remaining 95% hydrolyzed to nonvolatile sulfur-containing compounds. Since these latter compounds have biological importance, additional studies were undertaken to identify the specific compounds formed. Enzymatic conversion in water slurry at an optimum pH of 5.9 yielded R-goitrin to the extent of 75% of the total crambe thioglucosides. Quantitative yields of thiooxazolidone based on the total thioglucoside value were not obtained; however, other tests showed rather conclusively that no other thioglucoside than the progoitrin type were present in significant amounts in the meal. Paper and thin-layer chromatographic studies with two different solvent systems failed to resolve other thioglucosides from rather pure fractions isolated by ion exchange. Conversion of the purified thioglucosides at pH 5.9 did result in a quantitative yield of thiooxazolidone. In addition an examination of the forms in which sulfur is bound in crambe demonstrated that all of the thioglucosides in this seed are of the type containing 1 mole of aglycone sulfur per mole of sulfate sulfur, a relationship true for the thiooxazolidone precursor.

Enzymatic Modification of Crambe Meals

Since there appeared to be only one major thioglucoside present in crambe, enzymatic conversion was expected to yield an isothiocyanate cyclizing readily to a nonvolatile thiooxazolidone. This expectation was essentially realized when some crambe meals were enzymatically hydrolyzed in large volumes of water. In studies using 3 grams of meal in 100 ml. of buffer the yield of the thiooxazolidone was observed to increase as the natural pH of the meal was increased to pH 5.9 after which there again was a decline. Thiooxazolidone formation was found to be complete in 15 minutes at 54° C. and in less than

3 hours at room temperature. The yield was not affected by temperature. Maximum thiooxazolidone yield in one sample accounted for three-fourths of the total thioglucosides, whereas in another sample it accounted for only one-third of the total. This latter seed was unusual also in exhibiting much poorer feeding results than other meals tested. A connection between these two observations is likely and the reasons for the decreased yield of thiooxazolidone are being sought.

Hydrolysis of crambe thioglucosides was even more complex under conditions of reduced moisture. The enzyme hydrolyzed meals fed to rats and chicks were produced by essentially the same procedure as that previously reported with mustard, i.e., enzymatic hydrolysis carried out at a 30% moisture level. The rate of thioglucoside hydrolysis appeared to be little different than that observed with large volumes of water; however, the formation of thiooxazolidone was reduced to a few tenths of a percent. Once formed the thiooxizolidone was quite stable indicating a difference in the type of conversion product formed. Possibly the thioglucoside was hydrolyzed differently under these conditions as in the formation of organic cyanide and sulfur or perhaps a binding reaction occurred between the isothiocyanate released from the thioglucoside with meal protein making cyclization to thiooxazolidone impossible.

Rat feeding studies conducted at WU showed that the enzyme conversion processes yielded meal that gave growth rates up to 80-90% of the control diet where the proportion of crambe meal in the feed diet reached as high as 30%.

Dry Heat Processing

The palatability and feeding value of crambe meal were improved by a heat treatment under dry conditions. Some changes in at least a portion of the thioglucosides appeared to occur under these conditions and undoubtedly other deleterious constituents were also affected. The chemical changes accompanying this treatment were much less evident and more difficult to determine than the physical changes in the meal which were beneficial to the defatting processes. Successful Filtration-Extraction depends upon high rates of oil extraction and cake porosity which can only be achieved through a cooking procedure. By adding little or no water to crambe flakes and heating to 212° F. the meal was cooled and "crisped" to produce a material adaptable to Filtration-Extraction. Benefits of this heat treatment were also realized in the direct solvent extraction of crambe. Unheated flakes in an immersion extractor produced "fines" which clogged screw conveyors, dusted disagreeably, and caused a high "fines" content in the miscella. Heating flakes containing 4% moisture to approximately 230° F. and then hexane extracting presented no processing problems and produced a better quality oil than obtained from the unheated flakes. Similar benefits of dry heating would be expected in the Pre-press Solvent Extraction process where flakes are first pressed and then solvent extracted in either immersion or basket type extractors.

Lamb Feeding Trials of Dry Heat-Treated Crambe meals

Lamb feeding trials of ED dry-heat treated as well as untreated crambe meals were carried out over a 71-day feeding period at the University of Illinois. The ruminant feeding experiment was begun in May 1963, and was designed to study nutritive value, gross toxicity, and acceptability.

The dry matter and nitrogen in the crambe meal diets were digested as well as in soybean meal diets and the digested nitrogen from crambe meal was used almost as effectively as was nitrogen from soybean meal. There was no outward manifestation of toxicity from crambe meal fed as high as 30-35% of the diet during a 71-day feeding period. At the 12% protein level, the crambe meals supported gains comparable to soybean meal during the last 48-day period. Heat treatment improved digestibility and weight gains. The problem of acceptability of the crambe meals was perhaps the most serious one from the standpoint of practical feed lot usage. Inclusion of molasses in the diet and heat treatment improved palatability. The data from this preliminary trial suggest that crambe meal would compare favorably with soybean meal as a source of nitrogen for ruminants if the acceptability of the meal could be improved. Cattle feeding trials were suggested as a natural follow-up to the sheep trials by the University of Illinois researchers.

Detoxification of Crambe Meal by Solvent Processes

Several solvents were evaluated for their ability to remove thioglucosides from crambe meal. In one series, defatted crambe meal was extracted with several aqueous alcohols, aqueous acetone, and water to remove thioglucosides intact. The percent of sulfur removed and the percent of meal solubilized for each solvent system was measured. On the basis of the sulfur removal data, 50 and 70% methanol were equally effective in removing essentially all of the thioglucosides from the meal.

Dry heat treatments combined with 70% methanol extraction of a defatted meal produced a high-quality crambe meal with high feeding quality. Palatability was good and no organ abnormalities after autopsy were found following the 90 days of rat feeding. Quality of the oil by this process was excellent and the oil was not contaminated with sulfur products which interfere with the hydrogenation characteristics of the oil.

The solvent extraction of "enzyme hydrolyzed" crambe meals was studied to evaluate this process and to find a solvent giving maximum sulfur removal with minimum solids loss. As in the extraction of intact thioglucosides, the removal of sulfur components from converted crambe meals was obtained and was influenced by the water content and the chain length of the alcohol solvent used. Better meals from the standpoint of feed quality, however, were obtained in the earlier studies where thioglucosides were removed before conversion.

Procedures in which full-fat crambe was directly extracted with aqueous alcohols, acetone, etc., were carried out. This removed the oil and thioglucosides simultaneously in which the indicated quality of the meal was good based on sulfur removal data. These procedures are not considered a complete process, however, since the oil contained the undesirable sulfur compounds.

Commercial-Scale Plant Run on Crambe Seed Successful

Thirty-six tons of crambe seed grown during the 1963 season in Wyoming, Montana, Oregon, Idaho, and Colorado were processed in February 1964, at Sidney, Nebraska.

The run, made under contract for NU by the Pacific Vegetable Oil Corporation, was carried out in its commercial 150 ton per day prepress-solvent plant. Processing conditions specified as a result of engineering studies at NU were successfully incorporated. Operating steps, carried out in two phases, are shown on pages 46 and 47.

Based on plant data, observations, and laboratory tests of the products, the main objectives were accomplished and a successful run was obtained. Valuable information was obtained on material handling and on conditions required for prepressing and solvent extraction of crambe.

Quality of the products appeared good based on the chemical analyses. Material balances showed that good product yields were obtained. Separate analysis of the prepress and solvent oils indicated that both oils were of high quality and could be utilized as a blend. Fatty acids and phosphatides content was higher in the solvent oil. Refining losses were consequently higher for this oil than for the prepress oil. An important accomplishment for this run rests in the fact that enzymatic hydrolysis was under good control and sulfur hydrolysis products did not get into the oil to inactivate the catalyst for hydrogenation purposes. Both prepress and solvent oils hydrogenated readily to iodine values of one or less. Heat treatment during the processing was not excessive and good amino acid composition in the meal was found by chemical analysis. Over 13 tons of toasted meal were produced in this run and were forwarded to the University of Nebraska for cattle feeding trials.

Cattle Feeding Trials of Crambe Meal Prepared in NU-PVO Cooperative Test Run

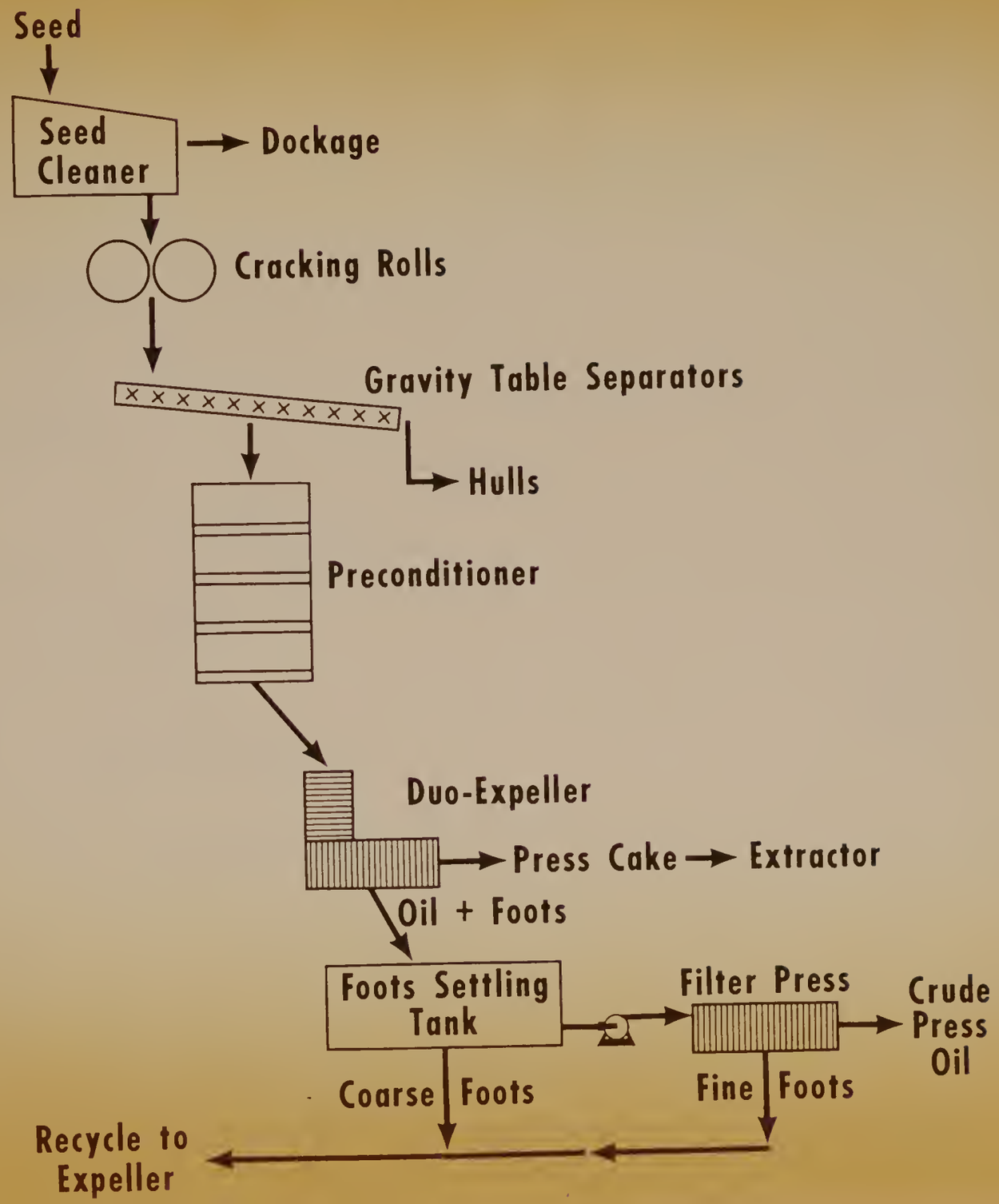
A preliminary report has been received from the University of Nebraska on the initiation of cattle-feeding studies with the large-scale prepared (PVO) crambe meals. Thus far, it appears that the cattle-feeding experiments are running somewhat parallel to our earlier lamb feeding work at Illinois (see above). There appears to be an initial resistance to feed containing only crambe meal because of an unpalatability factor. However, this may be overcome to a considerable degree by the blending in of molasses and incorporating crambe meal in a blend with other protein supplements. It appears that new process treatments to improve acceptability factors such as odor and taste are still needed.

Adaptation of Crambe to the Filtration-Extraction Process

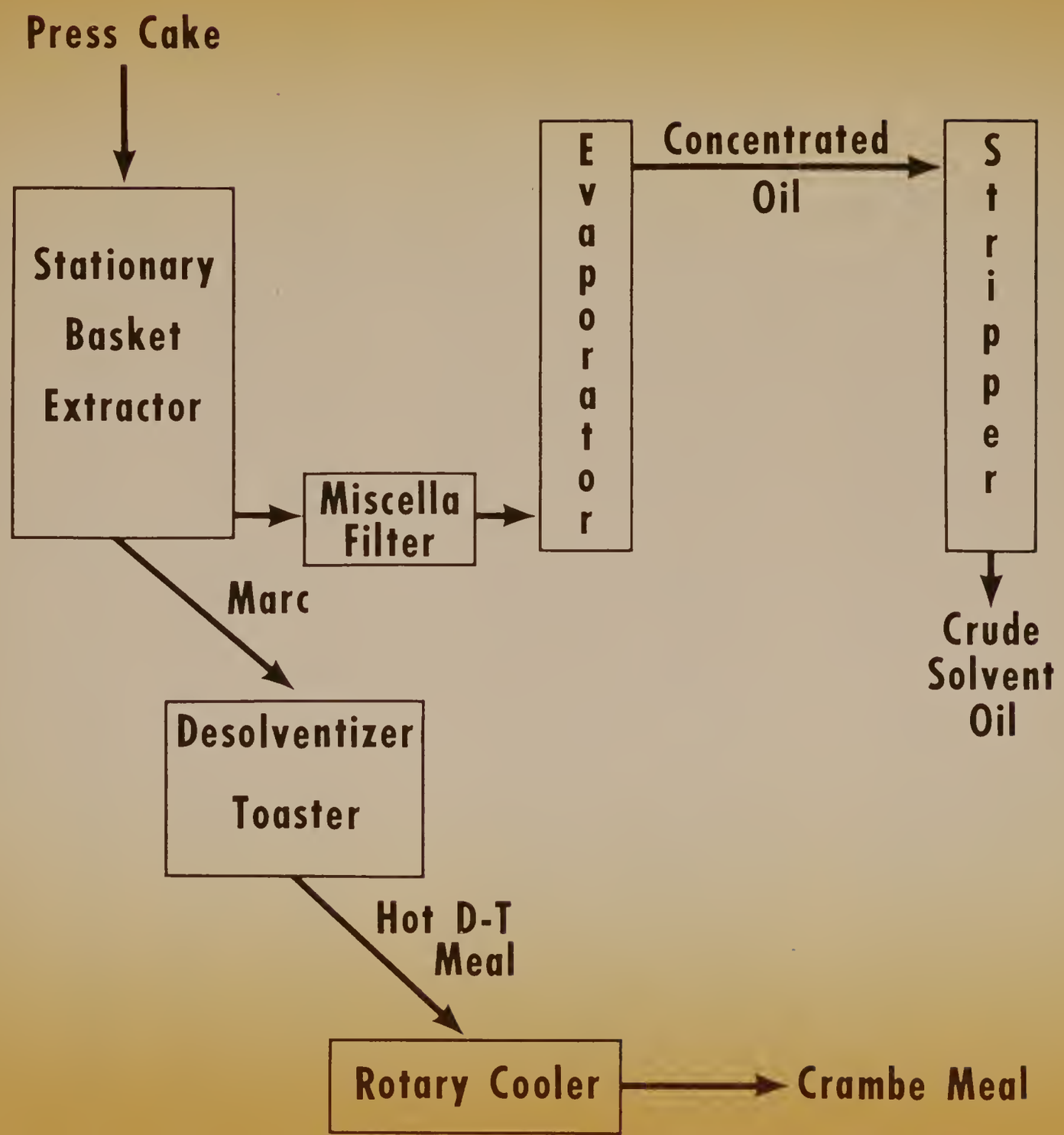
Crambe was found to be completely adaptable to the Filtration-Extraction (SU) process for recovery of the oil. Dehulled crambe flakes suitable for this process were prepared by cracking the whole seeds, dehulling, moisture tempering, and rolling into flakes. The hull content of crambe varied widely but generally constituted about 30% of the whole seed and was low in oil and protein. Removal of this fraction increased the final meal protein and reduced the quantity of meal to be extracted.

In the Filtration-Extraction process, a cooking step is required to promote release of the oil, produce a granular incompressible cake for rapid filtration, and prevent production of excessive fines in the meal. Cooking procedures were

Phase 1
Cleaning, Dehulling, Prepressing Crambe Seed



Phase 2
Secondary Oil Recovery



developed which could be adapted to crambe. In the procedure developed, flakes were heated to 185° F., moistened, heated to 212° F., and discharged from the cooker to cool and "crisp" to a granular meal suitable for rapid extraction. Rerolling the "crisped" meal through smooth rolls improved extractability. Good filtration rates were obtained and extraction results were similar to those published for other oilseeds. This procedure produced an oil free of sulfur contamination and hence suitable for hydrogenation. By a variation in the cooking procedure, moisture was added below 130° F. to activate the natural enzymes in the seed causing hydrolysis of the thioglucosides. Definite advantages in the meal's feeding value and palatability to rats were realized by this process. The oil, however, was contaminated with sulfur compounds which were not removed by conventional refining, bleaching, and deodorization. These compounds "inactivated" the nickel catalysts so that a commercial catalyst level resulted in only partial hydrogenation. For some projected uses of the oil where hydrogenation is not required this may not be a problem.

Progress and Future Emphasis

During the past period, several areas of crambe research have shown significant advances in overcoming the toxic effects of crambe meal to animals. Several basic approaches were followed independently in accordance with the chemistry of the process. A patent application for the enzymatic hydrolysis process, a low-cost detoxification procedure, has been applied for. Although rather complete detoxification procedures have been developed with solvent-processes, we feel that these processes may be too sophisticated and uneconomical. Future emphasis will continue on the evaluation of low-cost treatments in order to develop a simple and realistic process that can be accepted readily by the oil processing industry using standard or existing equipment as far as possible.

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Mustard Seed Processing: Bland Protein Meal, Bland Oil, and Allyl Isothiocyanate as a Byproduct, G. C. Mustakas, L. D. Kirk, and E. L. Griffin, Jr., J. Am. Oil Chemists' Soc., 39(8), 372, 1962.

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New Enzymatic Process Converts Mustard Seed to Valuable Meal and Oil Components, G. C. Mustakas, Chemurgic Digest V. 22, No. 1, January-February (1964).

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Mustard Seed Processing: Essential Oil Composition, L. D. Kirk, L. T. Black, and G. C. Mustakas, J. Am. Oil Chemists' Soc., 41(9), 599 (1964).

Submitted for Publication

Mustard Seed Processing: Improved Methods for Isolating the Pungent Factor and Controlling Protein Quality, G. C. Mustakas, L. D. Kirk, and E. L. Griffin, Jr.

Accepted by the Am. Oil Chemists' Journal.

In Preparation

Crambe Seed Processing: Filtration-Extraction on a Bench Scale, L. D. Kirk, G. C. Mustakas, and E. L. Griffin, Jr.

For publication in J. Am. Oil Chemists' Society.

Presentations

Crambe Seed Processing: Filtration-Extraction on a Bench Scale, L. D. Kirk, G. C. Mustakas, and E. L. Griffin, Jr. Presented at 55th Annual Spring Meeting of the Am. Oil Chem. Society, New Orleans, La., April 19-22, 1964.

Prepress-Solvent Extraction of Crambe: First Commercial Trial Run of New Oilseed, G. C. Mustakas, G. Kopas, and N. Robinson. Presented at 38th Annual Fall Meeting of the Am. Oil Chem. Society, Chicago, October 12-14, 1964.

Methods for Determining Thioglucosides in Crambe Abyssinica and Related Oilseeds, J. E. McGhee, L. D. Kirk, and G. C. Mustakas. Presented at 38th Annual Fall Meeting of the Am. Oil Chem. Society, Chicago, October 12-14, 1964.

Flavor and Oxidative Stability of Some Linolenic Acid-Containing Oils, H. A. Moser, C. D. Evans, G. C. Mustakas, and J. C. Cowan. Presented at 38th Annual Fall Meeting of the Am. Oil Chem. Society, Chicago, October 12-14, 1964.

Patents

U.S. 3,106,469, October 8, 1963--Method of Obtaining Detoxified Mustard Seed Products, G. C. Mustakas and L. D. Kirk.

Patents Filed

Method of Obtaining Detoxified Meal from Seeds Containing both Isothiocyanate and Thiooxazolidone, G. C. Mustakas and L. D. Kirk.

NU - Research on Vernonia

We have obtained approximately 4 pounds of Vernonia anthelmintica oil from EU to prepare vinyl esters of tetrahydroxy stearic acid. Mr. O'Donnell describes this research as follows:

Vernonia anthelmintica oil was reacted with acetone using boron trifluoride etherate catalyst to prepare the dioxalane derivative. The resulting product (S.E. = 347) was epoxidized yielding an epoxy derivative containing 3.8% oxirane oxygen. The epoxide was then reacted with acetone as above (S.E. = 376). Saponification and acidification gave an acid which was subjected to the acid-ester interchange reaction with vinyl acetate to produce the vinyl ester of the di-dioxalane derivative.

Treatment of an ether solution of the vinyl ester with concentrated hydrochloric acid at -30° C. failed to hydrolyze the dioxalane groups.

When the vinyl ester was treated with 1N dry hydrochloric acid in methanol a solid product resulted. Crystallization from acetone yielded a white solid (m.p. 119° C.).

I.R. analysis gave strong hydroxyl and vinyl ester absorption; no methyl ester was noted.

Elemental analysis (vinyl tetrahydroxy stearate)

Found	Theory
63.28% C	64.14% C
10.53% H	10.23% H

The low elemental analysis could indicate the presence of the methyl ester which was not noted on the I.R. spectrum. This route appears to be the most promising one to date for preparing vinyl tetrahydroxystearate.

Progress Report
EU NEW CROPS RESEARCH

DECEMBER 4, 1964

ARS New Crops Meeting
Peoria, Illinois

REPORT OF

EASTERN UTILIZATION RESEARCH AND DEVELOPMENT DIVISION

AGRICULTURAL RESEARCH SERVICE

UNITED STATES DEPARTMENT OF AGRICULTURE

ON

NEW CROPS RESEARCH

DECEMBER 4, 1964

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COVER: Copy of plate No. 515A from Kirtikar, K. R. and Basu, B. D., "Indian Medicinal Plants," Indian Press, Allahabad, part III (1918).

I. INTRODUCTORY STATEMENT

Developmental research on seed oils containing epoxy fatty acid components (EU assignment) in the past two years has been directed towards processing Vernonia anthelmintica (L.) Willd. (ironweed) seed; this has included refinement of the seed oil and its main component, trivernolin. Emphasis on this phase of the work has been necessary in the absence of an engineering project for performance of service. These products have been required in bulk in order to supply EU and industry with Vernonia working materials for evaluation studies, especially in the fields of plastics and surface coatings. Rapid increase in industrial interest in Vernonia is to be expected now that limited quantities have been made available for commercial testing, especially since, as one large company reports, refined Vernonia oil and trivernolin compare favorably with epoxidized soya and epoxidized tallate ester as stabilizer-plasticizers for PVC formulations.

Chief obstacle to rapid progress of Vernonia as a new crop continues to be the slow procurement of agronomic data on growing and harvesting. Work on the chemical phases to increase industrial potentials for this plant appears to us to have been a waste of money and effort unless Vernonia can be economically grown within a reasonable length of time.

We strongly believe that: UNTIL THERE IS REASONABLE ASSURANCE OF AGRONOMIC SUCCESS, ONLY PRELIMINARY RESEARCHES BE CONDUCTED ON ANY NEW PLANT OF INTEREST.

Change in emphasis has recently been made towards organic preparative work by way of synthesis of new compounds from vernolic acid, trivernolin and 12,13-dihydroxyoleic acid, the components readily available from Vernonia seed. Compositional studies are yet to be made on the unsaponifiables in the oil, on the meal, and on leaf material.

II. PROCESSING VERNONIA SEED

A. At EU Using Indian or Pakastani Seed.

Summary Statement

Vernonia anthelmintica (L.) Willd. (ironweed) seed from Pakistan or India produced 22-28% oil rich in trivernolin, the glyceride of 12,13-epoxyoleic acid, when the highly active lipolytic enzyme was properly controlled. Trivernolin was obtained from the seed as a major end product without isolation of the oil in yields of 55-60% of the weight of the oil present. Control of lipolysis was achieved, either by a rapid extraction technique where whole seed was flaked as a slurry made of seed and extracting solvent (petroleum naphtha, bp 35-59C), or by autoclaving the seed prior to flaking or grinding. An improved quality oil was produced by removal of the major portion of the unsaponifiable material; this upgraded the oil by increasing the epoxy lipid content, decreasing the iodine value and reducing the amount of color and odor.

REPORT: EU PROCESSING

Small-Scale Processing of *Vernonia* Seed. Small-scale operations were carried out with *Vernonia* seed working for optimum conditions of extraction. Methods of communition of seed consisted of cutting in a Ball and Jewel Shredder or Wiley Mill, flaking in a Lauhoff Rolling Mill, pulverizing in a Fitzpatrick Comminuting Machine, and cutting in the presence of solvent in a Waring Blendor. Details of these experiments are to be found in a recent publication (6) and previous publications referred to in (6). The Waring Blendor technique at 4°C proved to be the most effective procedure and this, with modifications, became the preferred analytical procedure for determination of the percentage oil in the more than 500 accessions of *Vernonia* seed examined to date.

The Waring Blendor experiments serve to demonstrate that optimum extraction (see the selected data of Table I) occurred at the temperature range 4-8°C

TABLE I

Waring Blendor Extractions of *Vernonia* Seed, 27.2% Oil

(46.0-g, mfb, Samples. Eight 400-ml p.n. extractions)

Experiment No.	Extraction Time	Extraction Temp	Oil Quantity		Oil FFA	Oil Oxirane oxygen
	hr	°C	g	%	%	%
1[a]	1.5	25	12.1	26.3	1.4	3.88
2	6.0	25	11.8	25.8	3.6	3.86
3	6.0	4-8	12.8	27.9	1.3	3.92

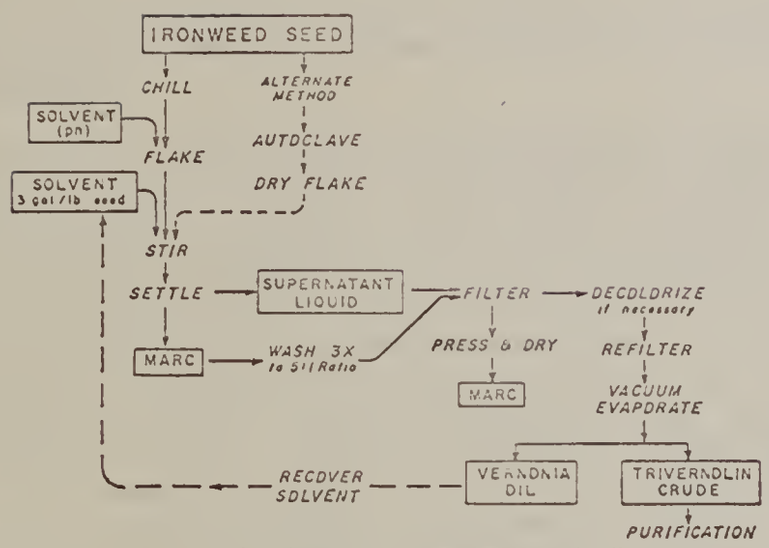
[a]Eight 200-ml p.n. extractions

with respect to quantity and quality of oil, the enzymatic activity being reduced to a minimum at the lower temperature; also haste in extraction in order to prevent lipolysis was not necessary at this temperature.

No great loss in efficiency was incurred if extraction were carried out rapidly at room temperatures as shown by data in Table I. In these experiments 50.0-g (46.0-g mfb) samples of whole seed (oil by exhaustive Soxhlet extraction, 27.2%) were used. Seed and p.n. (petroleum naphtha, Skellysolve "F") were ground together at high speed for 3 min. periods; the mixture was allowed to settle, and the supernatant liquid was decanted through a K-5 filter pad. In the first experiment each of 8 extracts was worked up independently for oil (breakdown not shown in Table I) which demonstrated that about 7 extracts had to be made to obtain all of the oil.

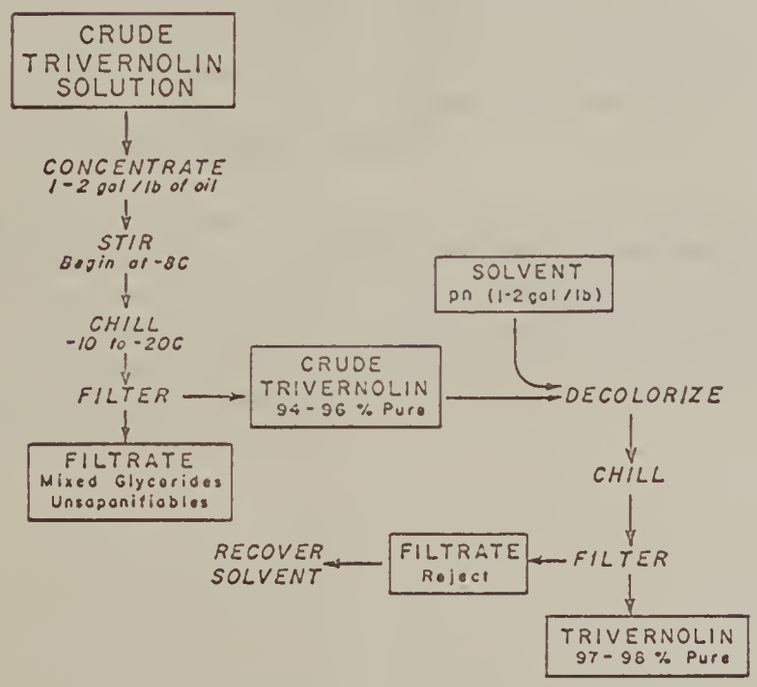
Larger-Scale Processing of *Vernonia* Seed. Larger-scale experiments were designed to duplicate Waring Blendor techniques as closely as possible with the equipment available. Procedures may be followed by reference to schematic Figures 1 and 2. In the small-scale experiments it had also been found that lipolytic enzyme activity in *Vernonia* seed could be controlled by heat inactivation, preferably by autoclaving under optimum conditions (6). In the

FIGURE I



Production of *Vernonia* seed oil.

FIGURE II



Isolation and purification of trivernolin.

semi-pilot plant operations both procedures, "rapid technique" and "autoclaved seed", were tried for production of trivernolin-rich oil and for trivernolin using 10-60 pounds of seed. Trivernolin was obtained directly without isolation of oil. Discussion of extraction is therefore under four headings as follows:

1. Rapid technique for oil. Three experiments presented in Table II illustrate this procedure.

Experiment 1 was included to show that seed flaked dry at room temperature could not be handled rapidly enough to prevent objectionable lipolytic action. In this experiment 58.5 lb. (26.6 kg with 7.7% moisture content) of Vernonia seed (oil by exhaustive Soxhlet technique, 22.0%) was flaked dry at a working temperature of 25-30C. The flaked seed coming off the mill rollers dropped immediately into a tank containing the p.n. extracting solvent. The mixture was diluted to 30 gal., stirred for about 3 minutes, allowed to settle for about 3 minutes and the supernatant extract pumped off through the filter press to the evaporators; the solvent was removed at once. A second extract was made immediately using 25 gal. of solvent and the same procedure; data on these and additional extracts are shown in Table II. Most of the oil was extracted in the first three extracts. However, the oil was not of satisfactory quality as indicated by the free fatty acid percentages of the extracts shown in Table II.

In experiment 2, 10 lb. (4.53 kg with 8.0% moisture content) of Vernonia seed (oil 27.2%) was wetted with about 2 to 3 gal. (sufficient to make a slurry) of p.n. This wet mixture was flaked and allowed to drop into the receiving tank containing solvent and the extracts handled as described in experiment 1. Most of the oil was obtained in the first two extracts, 30 and 5 gallon, respectively; about 3.5 gal. of p.n. per lb. of seed. Oil from the first extract was highly satisfactory with respect to quantity, low free fatty acid and high oxirane oxygen content. The lower temperature (10-16°C) supplied by winter weather in an unheated building was conducive to retarded enzyme activity in agreement with the small-scale Waring Blendor experiments.

In experiment 3, the second experiment was repeated with the exception that following dilution after flaking, the mixture was transferred to a steam-jacketed tank with a false bottom and heated during recycling for a period of three minutes. Extracts 2 - 4 were made with fresh boiling p.n. in similar fashion, combined and worked up together. No advantages over the cold extraction were gained by the hot extraction procedure.

TABLE II

V. anthelmintica Seed Extractions. Rapid Extraction for Oil

Expt. No.	Quantity of seed	Moisture in seed	Oil in seed	Chief variable in procedure	Extr. No.	Solvent used	Oil				
							Quantity		FFA as epoxy-oleic	Oxirane oxygen	Iodine value (Wijs)
	kg	%	%			gal	g	%	%	%	
1	26.6	7.7	22.0	Seed (dry) flaked at 25-30°C	1	30	4187	17.3	3.4	3.88	104.2
					2	25	942	3.9	10.1	3.82	105.1
					3	25	131	0.54	21.4	3.71	106.5
					4	25	55	0.23	26.1	3.58	109.0
					5	15	20	0.08	31.3	3.29	105.3
					Total	120	5335	22.0			
2	4.53	8.0	27.2	Seed (wet) flaked at 10-16°C	1	30	899.9	21.6	1.0	3.90	107.0
					2	5	73.2	1.8	3.9	3.84	105.0
					3	3[a]	22.5	0.54	4.1	2.60	84.9
					4	7[a]	37.3	0.89	5.3	3.42	99.5
					5	6[a]	21.3	0.51	6.8	2.78	90.6
					Total	51	1054.2	25.3			
3	4.53	8.0	27.2	Repeat Expt. 2 using hot extraction	1	30	895.4	21.5	1.1	3.90	104.4
					2-4	20[a]	100.6	2.4	4.5	1.92	70.6
					Total	50	996.0	23.9			

[a]New p.n. used; contaminated with kerosene.

2. Autoclaved seed for oil. A single experiment (Table III) has been

TABLE III

V. anthelmintica Seed Extractions. Autoclaved Seed for Oil.
(10.2 kg of seed containing 8.0% moisture, 27.2% oil)

Extraction No.	Solvent gal	Oil[a] Quantity g	Quantity %	FFA as epoxyoleic %	Oxirane oxygen %
1	20	1673.4	17.8	0.8	3.85
2	5	353.5	3.76	1.0	3.86
3	5	121.5	1.29	1.2	3.82
4	5	69.0	0.73	1.4	3.72
5	5	59.7	0.64	1.4	3.82
6	5	52.0	0.55	1.6	3.77
7	5	25.4	0.27	1.8	3.73
8	7	56.2	0.60	1.4	3.74
9[b]	5	52.5	0.56	3.8	3.27
10[b]	5	47.0	0.50	3.1	3.53
Total	67	2510.2	26.7		

[a]Iodine values were obtained but were not recorded since they were between 103 and 105.

[b]Marc re-extracted after 7-day storage.

selected for illustration in which optimum conditions for autoclaving the seed were used. The 22.5 lb. (10.2 kg, moisture 8.0%, oil 27.2%) of whole autoclaved Vernonia seed was flaked (not wetted with solvent) in the rolling mill equipped with automatic feed on the day following the heat treatment. Extracts were made in the manner described for the "rapid technique" using the quantities of solvent shown in Table III. Data for eight extracts showed that lipolysis was adequately controlled during the operation. Since exhaustive extraction had not been obtained two additional extractions of the marc were made one week later. Small quantities of oil were obtained with increased free fatty acid content indicating either selective extraction or that the hydrolytic enzyme was not completely inactivated by autoclaving under the conditions of the experiment.

3. Rapid technique for trivernolin. One selected example of the preparation of trinvernolin using the same procedure as previously described under the heading "1. Rapid technique for oil" is given in Table IV. About 15 lb. (7.32 kg, moisture 8.0%, oil 27.2%) of seed was wetted with solvent before flaking and extracting at 10 - 16C. A 30-gal. extract was made and a quick 5-gal. wash of the marc was added to the extract. After filtering no solvent was removed; the entire solution containing the extracted oil was placed in the refrigerator at -20°C. During crystallization which started at -8°C., the mixture was mechanically stirred. After removal of the crude trivernolin by filtration at -20° C., the filter cake was washed three times by removing it from the filter-plate each time, slurried with fresh cold

TABLE IV

V. anthelmintica Seed Extractions. Rapid Extraction for Trivernolin[a]

(7.32 kg. of seed containing 8.0% moisture, 27.2% oil)

Extraction No.	Solvent gal.	From crop no.	Fractions obtained by crystallization at -20°C: Quantity g.	Quantity %	Oxirane oxygen %	Trivernolin purity %	FFA as epoxy oleic %	Iodine value (Wijs)
1	30+5	1	864.2	12.80	5.00	96.6	0.50	83.9
		2	53.9	0.80	4.79	92.5	0.43	84.6
2	30	1	74.1	1.10	4.76	92.0	14.7	86.1
3	40	1	46.9	0.69	4.96	95.8	39.0	85.1
		2[b]	28.8	0.43	4.57	88.9	0.74	83.5
		Residue	698.5	10.37	2.21	42.7	7.93	128.9
Total 105			1766.4	26.2				

[a]Yield based on oil, 56.3% (96.0% pure) (15.4% based on the seed) using extracts 1-3 for calculation.

[b]All mother liquors from extracts concentrated to 1/2 gal.

solvent, mechanically stirred, and refiltered. Concentration of the mother liquor and washings to 3 gal. produced a second crop of trivernolin (53.9 g) in addition to the original 864.2 g. Additional extractions of the marc were made to obtain exhaustive extraction of the trivernolin; the number of extracts made and the quantities of solvents used are given in Table IV. Extracts 1 - 3, when combined, gave a trivernolin yield of 56.3% of the weight of the oil present in the seed with a purity of 96.0% based on the oxirane oxygen percentage. The extract marked "residue" in Table IV was obtained by evaporation of all mother liquors and was composed chiefly of mixed fatty acid glycerides (for fatty acid composition of *V. anthelmintica* seed oil see reference 1) and unsaponifiable material.

4. Autoclaved seed for trivernolin. A single experiment illustrates the use of autoclaved seed in the production of trivernolin. Information on the seed and the quantities of solvent used are given in Table V. Flaking and extraction procedures were similar to those described under "2. Autoclaved seed for oil." However, only partial crystallization of trivernolin occurred (376.5 g. obtained) when the entire first extract of 35 gal. was chilled to -20°C. To obtain a second crop (311.9 g) the mother-liquor was evaporated to 1.5 gal. and chilled to -20°C. Concentration of the second crop mother-liquor produced 37.8 g. of trivernolin of inferior quality. The 20-gal. extracts, 2 and 3, of the marc were concentrated to 3 gal. before cooling.

TABLE V

V. anthelmintica Seed Extractions. Autoclaved Seed for Trivernolin[a]
(6.68 kg. of seed containing 8.6% moisture, 22.1% oil)

Extraction No.	Solvent gal.	Fractions obtained by crystallization at -20°C: From crop no.	Quantity g.	Quantity %	Oxirane oxygen %	Trivernolin purity %	FFA as epoxy oleic %	Iodine value (Wijs)
1	30+5	1	376.5	6.17	4.99	96.4	0.34	80.8
		2	311.9	5.12	4.83	93.3	0.38	80.6
		3	37.8	0.62	4.54	87.7	0.61	82.6
2	20	1	24.8	0.41	4.78	92.3	1.9	83.6
3	20	1	2.0	0.03	4.49	86.7	2.0	-
		Residue	588.2	9.63	2.02	39.0	2.3	148.8
Total	75		1341.2	22.0				

[a]Yield based on oil, 51.3% (95.0% pure) (11.3% based on the seed) using extracts 1 and 2 for calculation.

Production of Vernonia Oil of Improved Quality: The process used to obtain an enriched oil with respect to epoxy fatty components consisted of the freezing out of the lipid glycerides from a p.n. solution with a ratio of about 3 ml. of solvent per g. of oil at -60°C. The solids were removed by vacuum filtration at -60° C. and washed several times with minimal amounts of cold solvents; when melted at room temperature traces of solvent were removed in a rotating evaporator under vacuum. Table VI presents data obtained

TABLE VI

Crystallization of Lipid Glycerides of V. anthelmintica Seed Oil at -60° C. for Removal of Unsaponifiable Material

	Quantity g.	Oxirane oxygen %	FFA as epoxyoleic %	Iodine value (Wijs)	Unsaponifiable material %
Oil	1698	3.85	0.86	104.1	6.67
Precipitate	1511	4.39	0.52	87.8	1.20
Filtrate	188	0.29	2.4	190.0	53.0

in this operation. Improvement in quality of the oil is indicated by the increase in oxirane oxygen content from 3.85 to 4.38%, a decrease in the iodine value from 104.1 to 87.8 and a decrease in the quantity of unsaponifiable material from 6.67 to 1.20%. Compositional studies on the unsaponifiable material are in progress.

The results obtained in the supplemental small-scale extractions established the feasibility of the rapid extraction procedure for larger-scale operations and indicated the desirability of the use of lower temperatures to retard lipolytic enzyme activity; also, the superiority of the use of solvent-wetted seed in the crushing operation. Although flaking was the preferred procedure for crushing Vernonia seed, preliminary experiments with the "FitzMill" did indicate the possibility of the use of a mill of this type for large-scale production if lower oil yields would be acceptable.

In the larger-scale operations, the flaking technique, using whole seed wetted with solvent prior to rolling, produced the best yields of high quality oil. The lower temperatures supplied by the winter working conditions were also conducive to the inhibition of enzyme activity. No advantage was gained by the use of hot solvent following the flaking operation. The technique of flaking dry seed at room temperature, followed by rapid handling in the extraction steps, was not satisfactory from the standpoint of producing an oil with a low free fatty acid content. Even when the flaked seed was allowed to fall directly from the rollers of the mill into the solvent this was not successful in holding down lipolysis.

The process of autoclaving the seed to inhibit lipolysis prior to flaking was also a successful procedure in oil production as illustrated by the fractional extraction data obtained. The slight variation in the free fatty acid content and oxirane oxygen composition of the ten extracts may have been due to selective extraction of the epoxy fatty components. When the seed was autoclaved no haste was necessary in carrying out extraction operations.

In situations where trivernolin was the desired end-product the "rapid technique" offered several advantages over the autoclaved seed treatment: the yield of good-quality trivernolin was higher; less coloring material was extracted; and it was not necessary to concentrate the solvent before crystallization in order to obtain the product in good yield. The solubility of trivernolin has previously been reported as about 5 g. per gal. at -20° C.

No plastic formulation data are available on the evaluation of Vernonia oil where the major portion of the unsaponifiable material has been removed. As this process appreciably elevated the oxirane oxygen percentage, reduced the iodine value, and removed color and odor, it may have resulted in an improved product for the purpose stated. One industrial concern has reported favorably on this improved product (see "III. Distribution of Vernonia Products to Industry", last page of this section).

II. PROCESSING VERNONIA SEED (CONT'D.)

B. At the VioBin Corporation Using Pakistani Vernonia Seed.

Summary Statement

Seed purchased from Pakistan in 1963 was extracted (3000 pounds) in a small pilot plant under "purchase order" agreement by the VioBin Corporation at Monticello, Illinois. Following extensive up-grading of their products at EU, 135 pounds of refined trivernolin-rich oil and 90 pounds of purified trivernolin were obtained for industrial distribution.

REPORT: VIOBIN PROCESSING

The VioBin Corporation was recommended by several Eastern companies as having a pilot plant suitable for *Vernonia* seed processing on quantities as small as the 3000-pound EU supply. Corporation representatives claimed to have all the equipment necessary for the EU process but would substitute Fitzpatrick Mill comminution for the flaking procedure recommended by EU. This was allowed provided temperature control would be maintained during comminution but only after EU investigators checked a FitzMill at the Eastern Laboratory. The data which are presented in Table VII indicated that a mill of this type would be satisfactory.

TABLE VII

Extraction of "FitzMill" Comminuted *Vernonia* Seed

(417.3-g. samples, mfb, oil 26.4%)

Experiment 1. For oil using eight 3.6-liter portions of n-hexane at 4°C.

	Oil			
Extract No.	Quantity		Free fatty acids (FFA) calc'd. as epoxyoleic	Oxirane oxygen
	g	%	%	%
1-3	99.6	23.9	2.5	3.78
4-5	1.5	0.36	9.3	3.00
6-8	0.7	0.16	12.0	2.67
Total	101.8	24.4		

Experiment 2. For trivernolin using one 3.6-liter; two 0.9-liter portions (combined) of n-hexane at 4°C.

	Trivernolin				
Crop No.					Purity %
1	45.4	10.9	0.6	5.04	97.4
2	8.2	2.0	0.7	4.70	90.8
Residue	34.0	8.1	5.3	2.03	39.2
Total	87.6	21.0			

An acceptable yield of 23.9% compared with the analytical figure of 26.4% with oil of equal quality was obtained. Also the trivernolin prepared from seed comminuted in this fashion was of good quality (97.4% pure). We immediately presented these data to VioBin and informed their chief chemist that the temperature must be controlled and under no circumstances could the seed be frozen or the oil yield would drop from 24% to 16%.

Agreement to use VioBin facilities was made on a "purchase order" basis for batchwise extraction procedure to require not more than 20 working days, 1000 pounds of seed to be processed for oil, 2000 pounds for trivernolin of not less than 95% purity.

Before shipment seed was precooled at EU and shipped by motor truck under refrigeration at 36° C.

In the presence of two EU consultants communition of seed was started August 19, 1963. (Seed had been purchased at 40 cents a pound from Pakistan through the assistance of Dr. Quentin Jones.) Alarm was expressed that the VioBin "FitzMill" was not equipped with a cooling device. Small amounts of dry-ice were being fed to the mill with the 36° F. seed to prevent overheating. If care is exercised and constant thermometer checks are made this is satisfactory. The first laboratory check on the oil extracted demonstrated the presence of only 16% oil in the seed indicating that the seed was being frozen in communition. This proved to be the case with 600 of the 1000 pounds having gone through the mill; temperatures had been ranging from -10° to +10° C. An immediate shift was therefore made to the EU autoclave process (6) and the remaining 2400 pounds of seed was autoclaved batchwise. A low yield of oil was therefore to be expected.

By August 23 the processing was approaching competion under supervision, miscella had been placed under refrigeration at -20° C. for crystallization of trivernolin and EU investigators returned to Philadelphia. Details of all that happened thereafter are not known. The oil sample submitted to EU later was satisfactory, the trivernolin (93%) was not. Insistence was made that the latter be recrystallized. On September 24 word was received that this had been done and oil and trivernolin (96% pure) were ready for shipment. These were picked up by Emory Motor Express for return to EU under refrigeration on October 1. The material was lost in transportation. Three weeks later on October 21, it was found in an abandoned trailer on a South Philadelphia lot without benefit of refrigeration.

Except for low yield the VioBin Vernonia oil was satisfactorily refined as described (6) without incidence, yield 135 pounds of excellent quality, oxirane oxygen 4.25%, free fatty acids 0.07%, iodine value (Wijs) 87.2%, Gardner number less than 1.

About four months later following continuous effort the trivernolin had been rid of impurities such as polymerized material and carbon (present from poor filtration technique at VioBin). The EU purified trivernolin (it was of only 91% purity when received from VioBin) was then of good quality (97% pure); oxirane oxygen 5.02%, free fatty acids (as epoxyoleic) 0.18%, iodine value 81.0%, Gardner number of 1.

About one-half of the VioBin oil and one-half of the trivernolin prepared have been supplied to Industrial Service Laboratories for evaluation studies, especially in the field of plastics and surface coatings.

II. PROCESSING VERNONIA SEED (CONT'D.)

C. At the Angola Soya Company Using Domestic Vernonia Seed.

Summary Statement

The first domestic seed (1963 crop) has been processed under EU workers' supervision at the Angola Soya Company at Angola, Indiana. This company made no charge for its services. The operations were performed rapidly in one working day without modification of equipment; no lipolysis of triglycerides occurred. Although the quantity of usable seed available was only 750 pounds, a much larger quantity could have been processed in this small plant equipped with automatic controls in the same length of time. This operation provided 93 pounds of refined oil, 10 pounds of purified trivernolin, and 8 pounds of unsaponifiables.

REPORT: ANGOLA PROCESSING

Upgrading Angola Seed for Processing. During the analysis of samples of Vernonia seed grown in test plantings throughout the country in the 1963 season, it was noted that there was a correlation between apparent seed density and quality, with the more dense seeds being higher in oil and oxirane oxygen content and lower in free fatty acids. Attempts were made to fractionate seeds on the density basis first by floatation in water, then by air elutriation. A seed sample which contained 23.7% oil, 3.38% oxirane oxygen and 8.55% free fatty acids (calculated as vernolic acid) was placed in water and those seeds which floated were separated by decantation. The two fractions were dried and analyzed with the following results:

	Oil	Oil	
		Oxirane Oxygen	FFA
	%	%	%
light fraction	19.8	3.08	12.63
heavy fraction	31.3	3.69	1.82

This showed that seed lots could be upgraded materially by removing light weight, immature seeds. Because separation by floatation in water was not practical for large lots and because it did not provide any latitude in the fractionating process, a small laboratory air fractionating column was constructed from a piece of glass pipe 2 in. in diam. and 4 ft. long.

One hundred-gram samples of two bulk shipments of seed A and B were placed in the laboratory air fractionator and separated into three fractions by gradually increasing the air velocity through the vertical glass pipe. The data for the original seed samples and three fractions are shown in Table VIII.

The goal was to obtain material for the commercial seed processing test which contained more than 25% oil with over 3.6% oxirane oxygen and less than 2% free fatty acids. The heavy fraction from sample A, which accounted for a little over 50% of the whole, had satisfactory oil and oxirane contents but was higher than desirable in free fatty acids. Sample B failed to yield a fraction suitable in either oxirane or acid content. It should be noted that these seed samples were lower in quality than those grown in some areas of the country, notably Georgia

and Kentucky, as shown in the recent report by White and Haun on the results from the 1963 test plantings.

TABLE VIII

Results of Vernonia Seed Fractionation by Laboratory Air Separator

Sample	Approx. Quantity	Oil[a]	Oil: Oxirane Oxygen[b]	Oil: FFA[b]
	g.	%	%	%
Original A	100	20.5	3.44	11.6
light fraction	33	12.1	2.80	25.2
medium fraction	11	18.7	2.97	19.5
heavy fraction	56	28.0	3.63	6.6
Original B	100	19.6	2.46	25.8
light fraction	15	2.9	1.58	36.8
medium fraction	60	15.0	2.49	27.7
heavy fraction	25	28.0	3.29	16.3

[a] Percent oil in seeds, mfb

[b] Percent oxirane oxygen and FFA (as vernolic acid) in extracted oil.

Based on the results of tests with the laboratory air fractionator, the Philadelphia Seed Company, commercial seed cleaners, was asked to attempt to separate two bulk lots of approximately 1500 and 580 pounds into light and heavy fractions in the hope that the heavy fractions would be of good enough quality to use in a commercial plant extraction test run.

Results of the fractionation in the commercial seed cleaning equipment are shown in Table IX. Because the first heavy fraction from sample A was

TABLE IX

Results of *Vernonia* Seed Fractionation by Commercial Seed Cleaning Equipment

Sample	Approx. Quantity	Oil	Oil: Oxirane Oxygen	Oil: FFA
	lb.	%	%	%
Original A	1500	20.5	3.44	11.64
light fraction-1	660	9.9	2.81	20.72
		(18.52)[a]	(3.18)	(14.1)
heavy fraction-2	840	25.3	3.49	8.89
light-2a	250	18.4	3.04	16.8
		(24.2)	(3.42)	(8.70)
heavy-2b	590	26.6	3.59	5.34
Original B	580	23.7	3.38	8.55
light fraction	330	9.5	2.67	14.19
		(17.8)	(3.06)	(10.0)
heavy fraction	250	28.7	3.57	4.35

[a] Data in parenthesis is % in total seed calculated from wt. and % of two fractions.

too high in free fatty acids, it was subjected to a second fractionation making approximately a 2 to 5 split. As the results in Table II show, there was some improvement in the quality of the second heavy fraction but the acid content of the oil was still high.

Brief Description of Angola Equipment Used in Processing. The equipment used was originally designed to process soybeans by continuous solvent extraction at a rate of 400 pounds per hour. Due to economic conditions the plant is now used for specialty extraction work such as processing wheat germ, sunflower seed, and pilot plant operations. Figure 3 shows flow diagram of the process.

In this plant raw material is usually received in bags which are emptied into a hopper from which it is conveyed into the flaking rolls, or directly to the extractor. A screw conveyor leading from the receiver hopper is used as a variable speed feeder. This conveyor is steam-jacketed so that the raw material may be tempered before flaking if desirable. *Vernonia* seed was not tempered. The flaking rolls consist of a set of smooth rolls, 18 inches in diameter and 18 inches long.

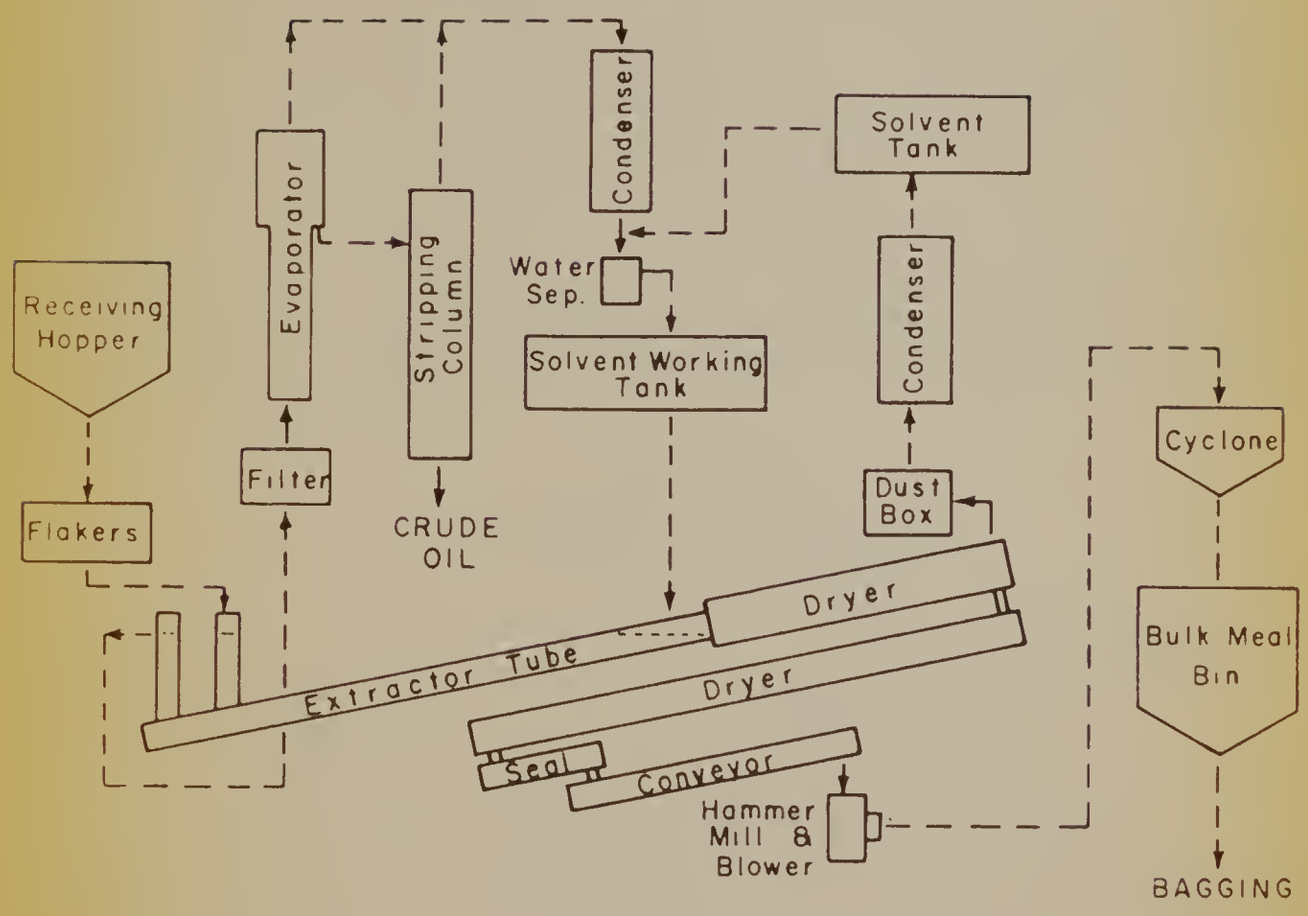
The Ford-type extractor in which the flakes are processed is an inclined tube, enclosing a ribbon conveyor and provided with two vertical standpipes at the lower end. One is for admission of fresh flakes and the other the miscella overflow. The flakes are carried upward, against a counterflow of solvent, which fills about 3/4 of the tube. The upper portion of the tube is steam-jacketed to effect preliminary solvent removal and immediately below is another steam-jacketed drier section to complete the separation of last traces of solvent. Connected to the bottom drier is a seal arrangement to keep solvent vapors and steam in the drier. From the seal, the meal drops into a screw conveyor which carries the extracted meal to a hammer mill. This ground meal is then blown into a cyclone and dropped into a bulk meal bin. The meal can be bagged as is or run through a burr mill and sifter to give a flour, 95% through an 80 mesh screen. This is then bagged and sewed ready for shipment.

The vapors from the meal drier are passed through a dust collection box and then to a tube and shell condenser which is connected to a solvent collection tank. The n-hexane and water from this tank are pumped to a solvent-water separator before the solvent is returned to a solvent working tank.

The miscella coming from the rear tower on the extractor is pumped through a leaf type filter to remove fines, and then into a long tube evaporator, which removes 80% of the hexane. The remainder of the hexane is separated from the oil in a disc and doughnut stripping column. Dry stripping steam is admitted to the bottom to aid in removing last traces of solvent. Solvent vapors from both the evaporator and stripping column are condensed in a common condenser. The condensate is piped to the solvent-water separator mentioned previously and then returned to the solvent working tank in the continuous gravity system. The crude oil from the stripping column is collected in a tank and can be further refined if necessary.

Prior to the use of this equipment for processing *Vernonia* seed, it had been used for wheat germ extraction but it had been thoroughly cleaned so that only a minimum amount of contamination would be encountered.

FIGURE III



Flow-Diagram Vernonia Processing.

Vernonia Oil Production and Refining. The Vernonia seed used consisted of 200 pounds of the 250 previously designated as "heavy fraction" of sample B (Table IX) and 559 pounds of the 590, the "heavy fraction 2b" of sample A (Table IX), a total of 717 pounds on a mfb basis analyzing 27.2% oil, the oil having an oxirane value of 3.58% and FFA (as epoxyoleic) of 5.07%.

In recent publications two methods for controlling lipolysis during Vernonia seed extraction have been offered, either a rapid extraction of freshly-flaked seed at room tmperature using n-hexane, or a heat inactivation treatment preferably by autoclaving prior to flaking. The former was used in this processing test being favored by winter weather conditions. The seed fed to the hopper was at 36° F., the temperature maintained in the plant during the extraction. However, the solvent temperature had increased to 60° F. by the end of the extraction. The seed was fed at a slower than normal rate, 100 pounds instead of 400 per hour, to handle the small batch more efficiently. About one hour was required for seed to pass through the processing operation ending up as oil and dried, bagged meal. On-the-spot analyses of miscella at intervals during processing for free fatty acid content of the oil showed it to be between 5.04 and 5.05% agreeing with one of 5.07%, the analytical figure previously mentioned.

A forerun fraction of miscella, containing 21.7 pounds of oil, was withdrawn to serve as final clean-out of equipment before starting collection of the main oil fraction. A tail fraction was obtained later in the final clean-up of equipment; this has not been refined. Table X shows the material balance obtained in the processing of Vernonia seed.

TABLE X

Vernonia Seed Processing

Material Balance

			Quantity	
			lb.	lb. (mfb)
Gross Seed		(IN)	759	717
Meal		(OUT)	487	469
Fines		"	60	56
Crude Oil	Forerun	"		22
	Main Fraction	"		149
	Tail Fraction	"		18
Total				714 (0.4% loss)

The output of 713 pounds is in close balance with the 717-pound input. The total of 189 pounds of oil obtained represents a yield of 26.3% of the weight of the seed on a moisture free basis as shown in Table XI.

TABLE XI

Vernonia Seed Processing

Oil Yield	%	Meal Analyses	Oil in %	Protein in %
From seed processing	26.3	Meal	2.2	12.5
By analysis of seed	27.2	Fines	1.3	56.3
Recovery	96.7			
Oil loss in processing (by difference)	0.9			
Oil loss (calc'd. from analysis of meal and fines)	0.3			

This compares favorably with the analytical figure of 27.2% and shows a recovery of 96.7%. Also as shown in this table, the 469 pounds of meal obtained had a fat content of 2.2% and the 56 pounds of fines, 1.3%. More exhaustive extraction of the seed would be desirable. The bulk of the meal was low in protein at 12.5% and the fines were high at 56.3%.

The main oil fraction containing 148 pounds of oil was retained in the form of a miscella in about 50 gallons of solvent. An aliquot containing 42.5 pounds of oil was removed for use in the preparation of trivernolin. The remainder of the main fraction miscella containing 106.5 pounds of oil was diluted to 55 gallons with n-hexane to eliminate air space. Refinement of this fraction was carried out at EU.

Refinement of Angola Oil. The procedure used in this refinement (6), consisted of the removal of unsaponifiable material by withdrawal of the solids, that is the mixed glycerides at -76° F. from a hexane solution, 70 gallons of hexane per 100 pounds of oil. The bulk of the unsaponifiables, which make up about 7% of the weight of the oil, remains in the filtrate and washings at low temperatures. This partially-refined oil was redissolved in hexane and washed free of acids with a dilute methanolic potassium carbonate solution. The hexane solution of oil was decolorized with a mixture of Filtrol No. 4 and Darco G-60. The refined oil obtained amounted to 75.3 pounds. The results of the analyses on the oil fractions are shown in Table XII.

TABLE XII

Vernonia Oil Analyses (Angola Process)

	Forerun		Main Fraction		Tail Fraction
	Crude	Refined[1/]	Crude	Refined[2/]	Crude
Oxirane oxygen (%)	3.57	3.84	3.56	4.09	3.24
FFA (%)	5.5	0.30	5.5	0.20	6.3
Iodine value (Wijs)	106.0	97.3	106.7	91.7	116.0

[1/] 75.3 lbs. [2/] 17.3 lbs.

The main fraction with an oxirane oxygen value of 4.09% and free fatty acid content of 0.2% had a bland odor and a Gardner number of less than one.

Preparation of Trivernolin (Angola). The 42.5-pound *Vernonia* oil aliquot reserved for trivernolin production was diluted to about 30 gallons with hexane. This solution was decolorized with Filtrol and Darco and the filtrate, bearing chiefly trivernolin, held at +10° F. overnight with slow-speed stirring during crystallization. The crystals of trivernolin were strained off on canvas at +10° F. and returned to EU for refinement. This was achieved by recrystallization from n-hexane as previously described (6). The yield was 56% based on the amount of oil present in the seed; its purity was 96% based upon the oxirane oxygen value.

Some of the refined *Vernonia* seed oil and the trivernolin have been distributed for commercial evaluation in plastic formulations. Quantities of both products have been reserved for the preparation of derivatives and evaluation studies at EU.

III. DISTRIBUTION OF VERNONIA PRODUCTS TO INDUSTRY

To date 135 pounds of Vernonia oil, 191 pounds of seed for extraction purposes, 204 pounds of meal for animal feeding tests, 30 pounds of trivernolin, and 18 pounds of unsaponifiable material have been distributed to outside agencies, chiefly industrial, for evaluation studies in the fields of plastics and surface coatings. The following is a list of agencies by name (other than those with amounts less than 25 grams) with the quantities of Vernonia products supplied:

Industrial

Name	V. Oil (lbs.)	Trivernolin (lbs.)	V. Seed for Extraction (lbs.)	V. Meal (lbs.)	V. Unsap. (lbs.)
Union Carbide	27	2	-	75	-
MSA Res. Corp.	-	2	5	75	-
ADM	11	2	1	-	-
Distillation Products	8	-	-	-	8
Vanderbilt Corp.	4	-	49	-	-
Ralston Purina	4	-	-	-	8
Keystone Chemurgic	4	-	-	-	2
~~Distillation Prod.~~	~~8~~	-	-	-	-
General Tire & Rubber	10	2	-	-	-
Westinghouse	2	2	-	-	-
Baker Castor	2	2	25	-	-
Cargill	4	2	-	-	-
Allied Chemical	2	-	-	-	-
A. E. Staley	2	-	-	-	-
Reichhold Chem.	2	2	-	-	-
Armour	4	2	-	-	-
Metal & Thermit	2	-	2	-	-
General Mills	3	2	-	-	-
Swift	2	2	10	-	-
W. R. Grace	2	4	5	-	-
Hollingshead	10	1	-	15	-
Sherwin Williams	2	-	-	-	-
Bale Bros. Res. Corp.	2	2	-	-	-
Argus Chem. Corp.	1/2	-	-	-	-
Kessler Chem. Co.	1/2	-	-	-	-
Dunlop Rubber Co.	1/8	1/8	-	-	-
Enterprise Paint Mfg.	1/2	-	-	-	-
General Foods	1/8	-	10	-	-
Midwest Res. Inst.	1	-	-	-	-
Ferrochemical	2	2	-	-	-
Atlantic Refining	-	-	10	-	-
FMC	-	1/4	-	-	-
Rietz Mfg.	-	-	25	-	-
Bauer Bros.	-	-	5	-	-
Spindle Top Res. Found.	-	-	2	-	-
Tucker (Consultant)	-	-	2	-	-

Federal, State & Others

Name	V. Oil (lbs.)	Trivernolin (lbs.)	V. Seed for Extraction (lbs.)	V. Meal (lbs.)	V. Unsapon. (lbs.)
NU	5	-	10	-	-
WU	2	1/8	-	39	-
DFA	1/2	-	-	-	-
Heiss, Munich	1/4	-	-	-	-
No. Dakota State Univ.	4	-	-	-	-
Univ. of California	1	-	-	-	-
Purdue Univ.	1/16	-	-	-	-
N. I. H.	1/16	-	-	-	-
Univ. of Conn.	-	1/4	20	-	-
CR	-	-	72	-	-
Hormel	-	-	10	-	-

A recent report furnished to EU by one of these industrial agencies in reference to Vernonia seed oil and trivernolin concluded:

"Both materials proved to be very interesting as stabilizing plasticizers for PVC, particularly the Trivernolin oil.

"Trivernolin compares favorably with epoxidized soybean oil for all permanence properties and light stability and shows a slight improvement over Peroxidol 780 for long-term heat stability. Both trivernolin and the Vernonia seed oil have low temperature properties which would be comparable to an epoxidized tallate ester such as Peroxidol 781."

IV. VERNONIA ENZYME STUDIES

Three publications have appeared since the last report (3, 4, 10) on Vernonia enzyme studies so that a full discussion will therefore be omitted from this report.

Work of this nature is now being continued cooperatively with the University of Connecticut and the University of Rhode Island. The following statements summarize these studies:

Vernonia anthelmintica (L.) Willd. seed was found to contain enzymes that were active only after the seed was ground. By deactivation of the enzymes, an oil rich in trivernolin (the triglyceride of epoxyoleic acid) was produced, and pure trivernolin was isolated. Acceleration of the enzyme activity altered the composition of the oil as evidenced by changes in free fatty acid content, iodine values, and oxirane oxygen (epoxy) content. Investigation showed that these changes were due, at least partially, to the conversion of epoxyoleic acid to (+)-threo-12,13-dihydroxyoleic acid, which was isolated in pure form. Pure (-)-threo-12,13-dihydroxyoleic acid was prepared by acetolysis of V. anthelmintica seed oil. Neither of these isomers had been obtained previously from mature V. anthelmintica seed.

Vernolic acid occurs as the triglyceride in the V. anthelmintica seed. Incubation of the seed produces a 1,3-divernolin. To determine whether the structure of trivernolin is responsible for the apparent secondary ester position specificity of the natural enzyme, trivernolin and triolein were incubated with pancreatic lipase and the free fatty acids and monoglycerides were determined after 5 and 15 minute digestion periods. The preponderance of 2-monoglyceride produced by the action of pancreatic lipase was interpreted to indicate that the structure of trivernolin was not solely responsible for the secondary position specificity of the V. anthelmintica lipase toward trivernolin.

V. EFFECT OF STORAGE ON EPOXY CONTENT OF V. OIL AND TRIVERNOLIN

Summary Statement

Accessions of Vernonia anthelmintica (L.) Willd. seed from India and Pakistan varied somewhat in the amount of oil that they contained, but the oils did not vary significantly in their epoxy content. Storage of the whole seed for periods

up to 3 years did not affect the quality of the oil, but the activity of the seed enzyme system seemed to increase with time. The data also show a rapid development of free fatty acid once the seed is ground. The epoxy content of low FFA Vernonia oil and trivernolin changed only slightly when these products were stored at room temperature for 6 months. However, the viscosity of the samples which were exposed to light increased greatly indicating changes in the physical nature of the products. Similar behavior was exhibited by both products when they were stored under nitrogen at 100° C. and by trivernolin at 4° C.

REPORT: STORAGE EXPERIMENTS

The products obtained from controlled utilization of the enzyme activity of Vernonia anthelmintica (L.) Willd. seed and the methods of deactivating and of activating the enzyme system have been investigated and discussed briefly in the previous report and in more detail in (3, 4, and 10). In these investigations it was found that the enzyme system was very highly active in crushed or ground seed and produced gross changes in the composition of the oil obtained from such seed. However, it was not known if the enzymes were active during prolonged storage of mature whole seed; if so, lipolytic activity in the whole, uncrushed seed would affect adversely the composition of the oil. Another unknown was the effect that storage had on the epoxy content of Vernonia oil and its chief component, trivernolin. This knowledge is essential since these natural products are potential stabilizers and plasticizers of poly(vinyl chloride).

Studies have been made which include: (1) a comparison of the composition of the oil obtained from several seed accessions; (2) the effect of storage of the whole seed on the composition of the oil; and (3) the effect of storage on the epoxy content of low free fatty acid (FFA) Vernonia oil and trivernolin. Also, some observations on the effect of storage of the whole seed on the activity of the seed enzymes have been made. More detailed accounts of these studies are to be found in (7).

Storage of Whole Seed. Seed was stored at 4° C. and at 27° C. (room temperature). At 4° C., the canvas bags containing seeds were put in plastic bags to protect the seeds from moisture. At 27° C., the seeds were stored in the canvas bags without any other protective covering.

Storage of Low FFA Vernonia Oil and Trivernolin. Low FFA Vernonia oil and trivernolin were prepared for storage in the following three ways: (1) Samples were sealed under a N_2 atmosphere; (2) samples were put in wide-mouthed loosely-covered containers that allowed the products to be exposed to the surrounding atmosphere; (3) samples to which a stabilizer, 0.3% tert-butylhydroquinone, had been added were also put in wide-mouthed loosely-covered containers. Samples from each of the above three groups were stored under the following four conditions: exposed to light at room temperature; not exposed to light at room temperature; at 100° C.; and at 4° C.

The data in Table XIII show that the only significant differences among seed accessions were in the moisture content and in the amount of oil in the seed. In general, higher yields of oil were obtained from seed grown in Pakistan than from seed grown in India. The analyses of the oils of the six seed accessions show no significant differences with respect to FFA and oxirane oxygen content and iodine values.

TABLE XIII

Comparison of Analyses of *V. anthelmintica* Seed Accessions

Origin and date rec'd.	Moisture when rec'd. %	Oil in seed (mfb)[a] %	Analysis of oil[b] FFA[c] %	Oxirane oxygen %	I.V. (Wijs)
India					
Feb. 1960	6.21	23.9	2.0	3.90	105.8
March 1961	7.80	25.5	2.0	3.90	106.3
Aug. 1961	9.20	23.3	2.1	3.95	104.4
July 1962	7.70	22.0	1.6	3.92	104.3
Pakistan					
Sept. 1962	8.04	27.2	2.0	3.95	103.4
June 1963	8.00	26.4	1.9	3.77	106.1

[a]Yield obtained by exhaustive extraction of ground seed. mfb - moisture free basis.

[b]Oil for analyses obtained by rapid extraction technique.

[c]Calc. as epoxyoleic acid.

Storage of the whole seed at 4° C. for periods up to 21 months and at 27° C. for periods up to 38 months did not affect the quantity or quality of the oil as shown in Table XIV.

TABLE XIV

Effect of Storage of Whole *V. anthelmintica* Seed on Oil Composition

Storage time mos.	Moisture %	Oil in seed (mfb) %	Analysis of oil FFA[c] %	Oxirane oxygen %	I.V. (Wijs)
6[a]	7.80	24.0	2.0	3.91	106.7
11[a]	7.26	23.8	1.9	3.92	104.2
21[a]	8.16	23.6	1.3	3.89	104.3
12[b]	6.21	23.9	1.9	3.90	105.0
31[b]	7.96	23.2	2.0	3.88	104.9
38[b]	7.50	23.8	2.0	3.95	106.8

[a]Stored at 4° C. [b]Stored at 27° C. [c]Calc. as epoxyoleic acid

Whole seeds were stored at 27° C. and at 4° C. for various times. After storage the seeds were ground; some of the ground seed was aged (exposed to atmospheric conditions at room temperature) before it was extracted and some was extracted immediately (aged 0 days). The data in Table XV show that the FFA did not develop in the intact seed during storage, but developed rapidly in the ground seed that was aged. In fact, this rapid development of FFA and the higher values obtained after the whole seeds were stored for a number of months indicates the possibility that enzyme activity increases with storage time. However, more complete study under carefully controlled conditions of storage, grinding and aging would be necessary to determine if this finding is significant.

TABLE XV

Effect of Storage of Whole Seed on Enzyme Activity

Whole Seed Stored Time mos.	Temp. °C.	Ground Seed Aged days	FFA[a] in oil %
0	-	4	14.5
11	4	0	1.6
21	4	0	0.5
21	4	21	49.3
21	27	0	1.3
21	27	4	22.8
21	27	21	47.9
38	27	4	27.4

[a]Calc. as epoxyoleic acid

The studies to determine the effect of storage on the epoxy content of low FFA *Vernonia* oil and trivernolin showed that the oxirane oxygen content cannot be used as the sole measure of the stability of these products. When low FFA *Vernonia* oil and trivernolin were stored at room temperature for six months, the oxirane oxygen content of both of these products decreased a maximum of 2% from the original 3.82 and 5.04%, respectively. The epoxy content of the samples that were protected from light did not decrease as much as that of the samples that were exposed to light. This would not have been considered relevant because of the very small maximum loss, but after six months the viscosities of the samples that were exposed to light had more than doubled their original values of 120.7 and 125.0 (at 26° C.) centistokes, respectively, while those of the oil and trivernolin that were protected from light had increased a maximum of 27% and 4%, respectively. Also a nitrogen atmosphere provided the most protection against loss of oxirane oxygen. In fact, the oil samples that were stored under nitrogen retained their original level of epoxy component.

As shown in Figures 4 and 5, storage of low FFA *Vernonia* oil and trivernolin for six months at 100° C. in a nitrogen atmosphere had little effect on their epoxy contents. Addition of a stabilizer, *tert*-butylhydroquinone (0.3%) also prevented any appreciable loss of epoxy from trivernolin. However, the viscosities of these samples increased greatly in six months to 300.4, 292.1, and 298.0 centistokes, respectively. The oil and the trivernolin that had no stabilizer added were completely polymerized, and the oil that had stabilizer added was too viscous

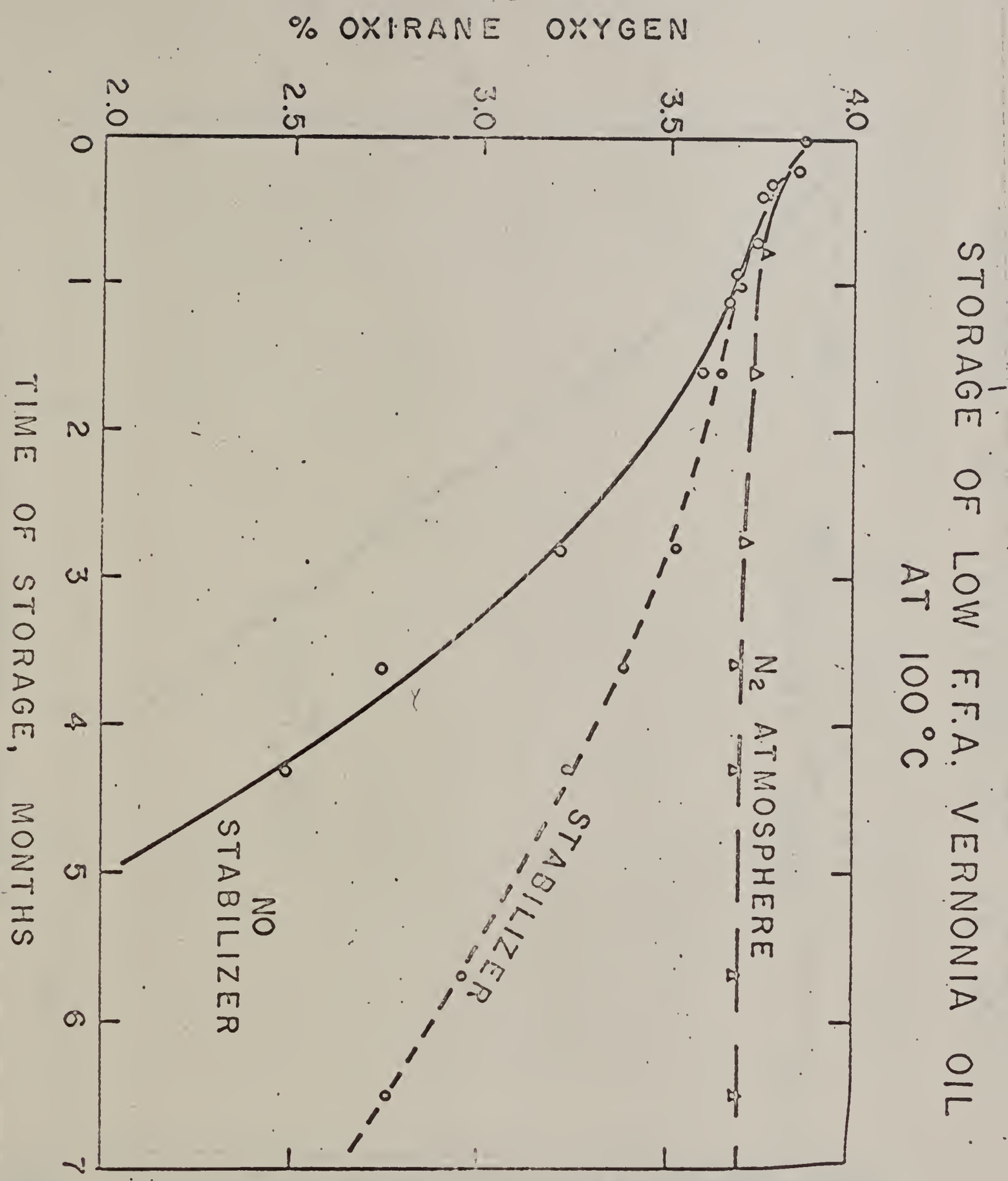


FIGURE IV. The effect on the oxirane oxygen content of low FFA Vernonia oil stored at 100C in the following ways: 1) sealed in a nitrogen atmosphere; 2) exposed to surrounding atmosphere; 3) stabilizer added, exposed to surrounding atmosphere.

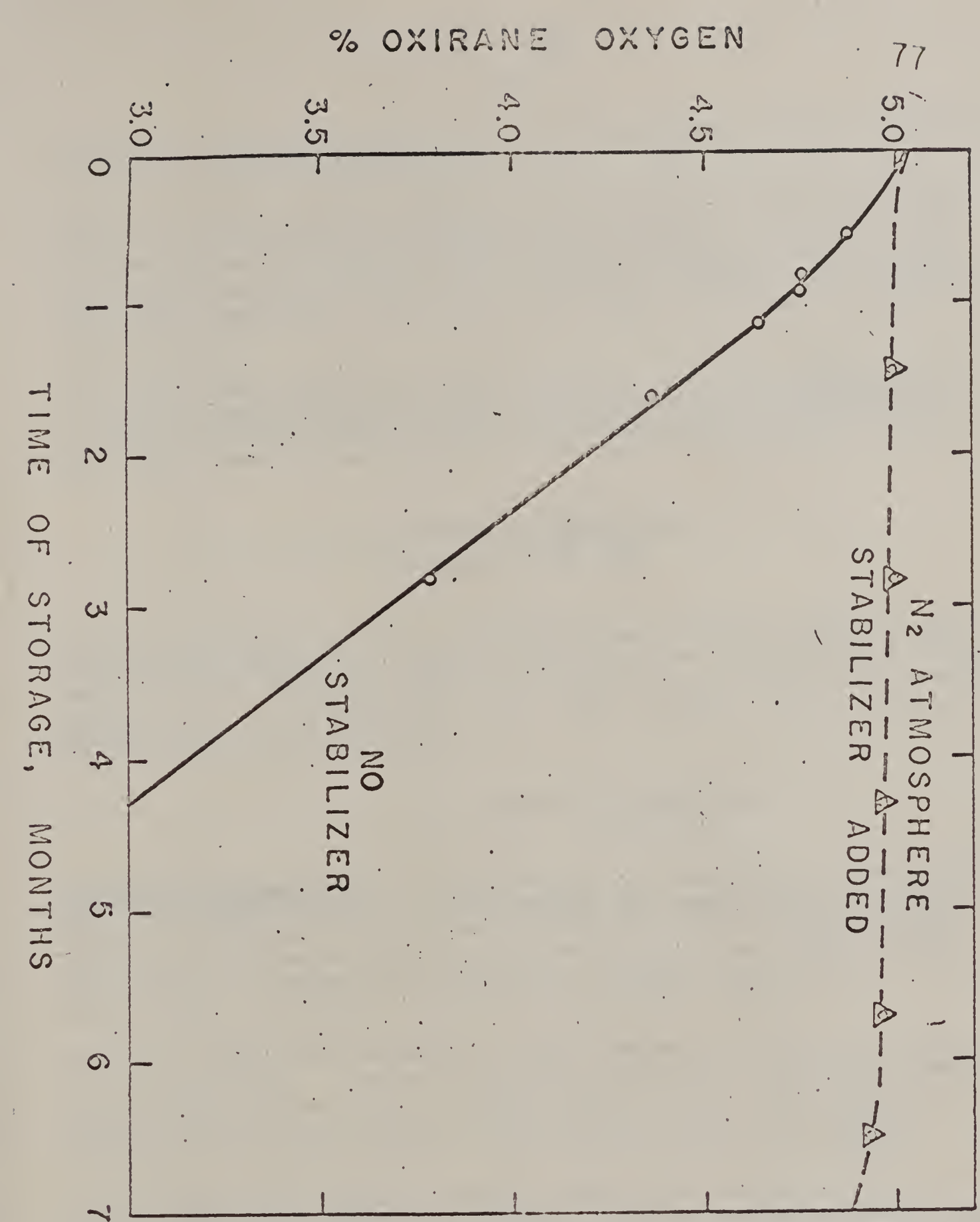


FIGURE V. The effect on the oxirane oxygen content of trivernolin stored at 100C in the following ways: 1) sealed in a nitrogen atmosphere; 2) exposed to surrounding atmosphere; 3) stabilizer added, exposed to surrounding atmosphere.

to measure. The oxirane oxygen content of these samples decreased considerably in six months (Figures 4 and 5).

The epoxy content of low FFA Vernonia oil was not affected adversely by storage at 4° C. for six months and had a maximum increase in viscosity of 21%. The epoxy content of trivernolin decreased only 2 to 4%, but the viscosities of the samples stored with and without stabilizer increased to 215.7 and 209.7 centistokes, respectively, while that of the sample stored under nitrogen increased to 168.5.

These results show that under certain conditions of storage Vernonia oil and trivernolin undergo changes in their physical nature that are not always indicated by the oxirane oxygen values. On the other hand, a decrease in oxirane oxygen content was accompanied by an increase in viscosity.

VI. STATEMENT ON VERNONIA MEAL EVALUATION STUDIES (WU)

Studies on Vernonia meal made at the Pharmacology Laboratory (WU) have indicated that good growth rate compared with control rats is made with autoclaved meal when fed at 20% dietary level for 98 days. Due to its high fiber content Vernonia meal may ultimately prove to be a more suitable feed for sheep and cattle than for swine and poultry.

VII. ANALYSES OF VERNONIA SEED

Analyses of Vernonia Seed. In cooperation with Chemurgic Crop Investigations, New Crops Research Branch, over 400 Vernonia seed samples grown in 1963 were analyzed for moisture and oil content and the quality of the oil from each sample was determined by oxirane oxygen and free fatty acid (FFA) analyses. These data were combined with yield data by Chemurgic Crops workers to evaluate the effect of different agronomic and geographic factors.

Analytical methods were developed to permit reasonably rapid routine analysis of the seed samples. Moisture analysis was performed by drying approximately 2-gram samples (ground to pass a 20 mesh sieve in a micro Wiley mill) in a vacuum oven for 4 hours at 80° C. with a dry air sweep. This procedure produced moisture values comparable with the toluene distillation method.

Oil was extracted by grinding a 10-gram sample with petroleum ether in a Waring blendor (high speed) for 3 minutes, filtering through a Soxhlet thimble and extracting the ground seed for 4 hours with petroleum ether. The combined Waring blendor and Soxhlet extracts were evaporated to about 50 ml., transferred to a volumetric flask, and diluted to 100 ml. 25-ml. aliquots were used to determine oil, oxirane oxygen and FFA. One aliquot was placed in a tared throw-away aluminum dish, the solvent evaporated on a steam bath and the oil weighed after a short vacuum drying period. Oxirane oxygen was titrated in a second aliquot using HBr in glacial acetic acid with crystal violet indicator and free fatty acids were titrated in another aliquot with 0.03 normal potassium hydroxide in 95% or "reagent" alcohol after adding thymol blue-cresol red indicator in isopropyl alcohol solution. The FFA were calculated as per cent vernolic acid.

Per cent saponifiables, calculated as trivernolin, were also determined for most samples by saponifying the samples after the free fatty acid determination. The data for saponifiables added little to the information obtained from the other analyses so this analysis will not be made on the 1964 samples.

VIII. PREPARATIVE WORK ON VERNONIA

This work is in its initial stages and may be summarized by stating that pound-quantities of epoxyoleic and 12,13-dihydroxyoleic acids have been prepared from trivernolin and Vernonia oil respectively for derivatives to be evaluated as plasticizers for PVC. A series of aliphatic esters of these acids and a series of chlorhydrin esters of vernolic acid are being prepared for evaluation.

IX. PLASTICIZER-STABILIZER STUDIES ON VERNONIA PRODUCTS

Preliminary crosslinking studies on Vernonia oil showed that a flexible thermosetting epoxy resin with moderate tensile strength could be obtained using triethylenetetramine. Other crosslinking agents have generally produced resins with poor physical properties.

A previous observation on the stability of trivernolin showed an unexpected increase in viscosity on storage at +2° C. and a decrease in the oxirane value. A study was made on trivernolin to determine whether the observed results were reproducible. Viscosity measurements were made periodically on samples of trivernolin stored in the presence of air at -29°, -16°, +2°, + 15° C. and room temperature. At the end of six months there was no measurable change in viscosity with the exception of the +2° C. sample which showed a decided increase in viscosity. A further study will be made over a longer period and will include samples stored in the absence of air in order to determine the cause of this behavior.

X. PUBLICATIONS, PRESENTATIONS AND PATENTS SUBSEQUENT TO LAST REPORT

1. "Epoxy Acids and Salts as Stabilizers for Vinyl Plastics," G. R. Riser, J. J. Hunter, J. S. Ard, and L. P. Witnauer. SPE Journal 19, 729-734, No. 8, August 1963.

   Presented: Am. Oil Chem. Soc. Meeting, New Orleans, La., 1962.

2. "A Satisfactory GLC Column for the Determination of Epoxyoleic Acid in Seed Oils," S. F. Herb, P. Magidman, and R. A. Barford. JAOCS 41, 222-224 (1964).

   Presented: Am. Oil Chem. Soc. Meeting, Toronto, Canada, 1962.

3. "*Vernonia anthelmintica* (L.) Willd. "Enzyme Studies. Conversion of Epoxyoleic Acid to (+)-*threo*-12,13-Dihydroxyoleic Acid," W. E. Scott, C. F. Krewson, F. E. Luddy, and R. W. Riemenschneider. JAOCS 40, 587-589 (1963).

   Presented: Am. Oil Chem. Soc. Meeting, Chicago, Ill., 1961, and Toronto, Canada, 1962.

4. "Lipolysis of Trivernolin by Pancreatic Lipase," J. Sampugna, R. G. Jensen, R. M. Parry, Jr., and C. F. Krewson, JAOCS 41, 132-133 (1964).

5. "*Vernonia anthelmintica* (L.) Willd. Highly Purified Epoxy Components from the Seed Oil," JAOCS 41, 134-136 (1964).
   Presented: Am. Oil Chem. Soc. Meeting, Atlanta, Georgia, 1963.

6. "*Vernonia anthelmintica* (L.) Willd. Extraction of Oil or Trivernolin from the Seed," C. F. Krewson and W. E. Scott, JAOCS 41, 422-426 (1964).

   Presented: Am. Oil Chem. Soc. Meeting, Minneapolis, Minn., 1963.

7. "*Vernonia anthelmintica* (L.) Willd. The Effect of Storage on the Epoxy Content of the Seed Oil and Trivernolin," W. E. Scott and C. F. Krewson, JAOCS (In Press).

   Presented: Am. Oil Chem. Soc. Meeting, Minneapolis, Minn., 1963.

8. "*Vernonia anthelmintica* (L.) Willd. Processing Ironweed Seed," C. F. Krewson, C. L. Ogg, F. J. Oelschlegel, Jr., Reginald Hale and A. H. Hale, JAOCS (In Press).

   Presented: Am. Oil Chem. Soc. Meeting, Chicago, 1964.

9. "*Vernonia anthelmintica* (L.) Willd. (+)- and (-)-*threo*-12,13-Dihydroxyoleic Acid," W. E. Scott, C. F. Krewson, and R. W. Riemenschneider, Chem. & Industry, 1962, 2038-2039.

10. "Preparation of (+)-*threo*-12,13-Dihydroxyoleic acid," W. E. Scott, C. F. Krewson, and R. W. Riemenschneider. U. S. Patent No. 3,139,387, June 30, 1964.

11. "Process for Obtaining Trivernolin," C. F. Krewson and W. E. Scott. PC No. 3946, SN No. 144,516. Allowed August 17, 1964.

12. "Process for Isolation of Divernolin and Trivernolin," C. F. Krewson, J. S. Ard, and R. W. Riemenschneider. PC No. 3926, SN No. 136,113. Allowed October 14, 1964.

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE

REPORT OF

SOUTHERN UTILIZATION RESEARCH AND DEVELOPMENT DIVISION

ON

NEW CROPS RESEARCH

DECEMBER 1964

During 1963 and 1964 development research was carried out at the Southern Utilization Research and Development Division on oils and fatty acids from Limnanthes douglasii; on petroselinic acid and petroselinic acid-containing oils from Umbelliferae; and on capric acid and capric acid-containing oils from Cuphea llavea.

Oils and Fatty Acids from Limnanthes douglasii

Many procedures were investigated for separation of the principal fatty acids from Limnanthes douglasii oil. As has been previously reported, these fatty acids are:

cis-5-eicosenoic acid	65%
cis-5-docosenoic acid	7%
cis-13-docosenoic acid	13%
cis-5-cis-13-docosadienoic acid	10%

Methods of obtaining the methyl esters of two of these acids in essentially pure form were developed. Methyl 5-eicosenate was obtained in purity of over 99% (by GLC analysis) by fractional distillation of the mixed esters through a Podbielniak column at reduced pressure. The higher boiling esters deteriorated on attempted fractionation and could not be separated by this means. A 12-gram sample of pure (by GLC analysis) methyl

5,13-docosadienoate was obtained from 450 grams of the mixed esters by crystallization from acetone (yielding 50 grams of 70%-pure product), followed by several fractionations of the mercuric acetate adduct from petroleum ether. This material and its analogous acid have been characterized. However, it does not at the present time appear economically feasible to separate these materials or the other C-22 components from Limnanthes oil for industrial uses. Therefore, it is planned to restrict continuing utilization studies to 5-eicosenoic acid and its derivatives; the total mixed acids and their derivatives; and the oil itself.

In the course of the above-described work, a rapid GLC analysis of the methyl esters of the component acids of Limnanthes oil was developed. A quick analytical method was needed, and the one developed will be useful in the continuing work with this oil.

The column employed to separate these esters had dimensions of one-quarter inch by ten feet, and was packed with 20% diethylene glycol succinate on Chromasorb W of 80-100 mesh. The column temperature was maintained at approximately 245° C. The thermal conductivity detector was maintained about 25° C. above the column temperature, and the injector part about 75° above the column temperature. Helium gas was used as a carrier. Flow rates of 40-70 milliliters per minute were effective in separating the Limnanthes methyl esters.

Analysis of Limnanthes douglasii seed grown in Alaska. A sample of Limnanthes douglasii seed, Lot No. PI 278170, grown in Alaska during the 1964 growing season, was received by SURDD and analyzed with the following results:

Moisture, 8.74%

Oil, 22.4% (dry basis, 24.5%)

Nitrogen, 2.84% (dry basis, 3.11%).

The fatty acid composition of the oil (by gas liquid chromatography of the methyl esters) was:

C-16 saturate,	0.2%
C-16 monoene,	0.2%
C-18 saturate,	0.1%
C-18 monoene,	1.5%
C-18 diene,	0.4%
C-20 saturate,	0.4%
C-20 monoene,	63.6%
C-20 diene,	0.6%
C-22 monoene,	21.3%
C-22 diene,	11.6%.

These results indicate that the Limnanthes douglasii grown in Alaska is very similar in composition to that grown elsewhere, which will facilitate utilization studies for its seed products.

Word has been received through Dr. I. A. Wolff, NU (his memo to Dr. Leo Goldblatt, SU, dated September 15, 1964), that the Alaska Agricultural Experiment Station has obtained seed yields of up to 3,000 pounds per acre (extrapolated from small plots) from Limnanthes in 1963 with the possibility of further increases in 1964. The Progress Report of the Alaska AES to the NC-7 Regional Technical Committee meeting dated

August 3, 1964, indicates considerable promise for Limnanthes douglasii in particular in Alaska. SU has requested, through Dr. John L. Creech, New Crops Research Branch, CRD, that arrangements be made with the Alaska AES for the production of 1,000 pounds of Limnanthes seed for larger scale processing and utilization studies.

Petroselinic Acid and Oils of Umbelliferae

Petroselinic acid-bearing oils, primarily from Umbelliferae, were again introduced into the program in 1962 as a result of a report from Crops Research that fennel had self-seeded or come up from root stock a second year in a Missouri planting and produced a reasonably good yield of seed. Further promising results with fennel were obtained at College Station, Texas (letter dated August 31, 1964, from Dr. Eli L. Whiteley, Texas A&M University to Dr. Leo A. Goldblatt, SU), where the estimated yield of seed produced by fennel in 1964 is 1,000 pounds per acre (extrapolated from small plots). Dr. Whiteley stated that a fall planting of fennel withstood temperatures of 12° F in January, 1964, and that he was planting one-half acre of fennel this fall to get a good test on the yield of fennel. He has supplied SU with 10 pounds of fennel seed from his 1964 harvest for analysis and possibly utilization studies.

Analysis of Fennel Seed grown in Texas. The fennel seed supplied SU by Dr. Whiteley from his 1964 harvest, Lot No. 0-928, were analyzed with the following results:

Moisture, 10.33 %

Oil, 9.55 % (dry basis, 10.65%)

Nitrogen, 3.04 % (dry basis, 3.39%).

The fatty acid composition of the oil (by gas liquid chromatography of the methyl esters) was:

C-16 saturate,	5.3%
C-16 monoene,	0.8%
C-18 saturate,	1.0%
C-18 monoene,	80.3%
C-18 diene,	12.2%
Unknown,	0.4%.

The C-18 monoenes, consisting of the methyl esters of oleic and petroselinic acid, were resolved by ozonolysis and reduction to the aldehydes, and comparison of the amounts of C_9 and C_{12} aldehyde by GLC analysis. The C_{12} aldehyde amounted to 90.0 %, indicating 72.3 % of the fatty acids to be petroselinic acid.

Utilization Studies with Petroselinic Acid. Petroselinic acid is being modified chemically in a continuing study of its chemistry and attempts to take advantage of its unique structure to prepare derivatives of potential industrial utility. The acid has been converted to petroselinylamine. The "ethomeen" (reaction product with ethylene oxide) and "duomeen" (reaction product with acrylonitrile, followed by hydrogenation of the nitrile group) derivatives have been prepared and submitted to Armour along with the amine for evaluation as corrosion inhibitors.

Reaction products of the Ritter reaction of petroselinic acid with several nitriles are being studied for possible utility. The 6(7)-aminostearic acid available from hydrolysis of these materials will be converted to a polyamide and the properties of this material determined.

The vinyl ester of the hexachlorocyclopentadiene adduct of petroselinic acid has been prepared and submitted to Dr. Marvel at the University of Arizona. He will investigate the properties of the copolymer of this material and vinyl chloride.

Investigation of the aldehydes resulting from reductive ozonolysis of petroselinic acid or its methyl ester as intermediates in the preparation of other materials of potential industrial utility is continuing. The hydrogen cyanide addition products of these aldehydes are currently being studied. In previous work, the aldehydes from reduction of the ozonide of petroselinic acid were allowed to react with formaldehyde and base to give the analogous trimethylol compounds, which may have a utility as polyols. Trimethylol undecane (2-decyl-2-hydroxymethyl-1,3-propanediol), from the lauraldehyde portion, was successfully separated in 35% yield. The trimethylol from the adipaldehydic acid portion of the molecule was not successfully separated because of its water solubility.

Decanoic Acid and Oil of Cuphea

In seeking uses for decanoic acid, the major component acid of *Cuphea llavea* and *C. ignea*, attempts were made to prepare 2-decenoic acid by dehydrohalogenation of 2-bromodecanoic acid with poor results. The action of sodium or potassium hydroxide in the presence of methanol, ethanol or propanol gave predominantly the 2-alkoxy compound corresponding to the alcohol and only small amounts of 2-decenoic acid. The presence of even small amounts of water in the solvent gave large amounts of 2-hydroxydecanoic acid as a byproduct. The use of quinoline as a dehydrohalogenation reagent

gave tarry mixtures from which only poor yields of 2-decenoic acid could be recovered. Though the dehydrobromination of 2-bromoalkanoic acids to yield the α,β-unsaturated acid is a common textbook reaction (see Fieser and Fieser, "Organic Chemistry," Reinhold Publishing Corp., New York, N. Y., 1956, 3rd edition, p. 58), no references reporting good yields from this reaction have been found in the literature. Since the 2-hydroxy acid can be readily prepared in high yield from 2-bromodecanoic acid, it is anticipated that any future work on development of a process for the conversion of decanoic acid to the α,β-unsaturated analogue will involve investigation of methods of dehydration of the 2-hydroxy acid. However, no such work is planned at the present as all the current effort on products from Cuphea is being applied to investigation of the oil per se to find possible utility for it as a plasticizer, in the production of alkyd resins, etc.

The following derivatives of decanoic acid were prepared and submitted to the U.S.Field Laboratory for Tung Investigations, CR, Bogalusa, La., for testing as growth regulators to retard the flowering of tung trees and avoid late frost damage:

Methyl decanoate

Neopentyl glycol ester of decanoic acid

Propargyl 2-bromodecanoate

2-Decenoic acid

4-(2-decenoyl)morpholine

4-(2-bromodecanoyl)morpholine

The latter five compounds were ineffective in tests on cut shoots, but methyl decanoate killed the buds on cut shoots even at a dilution of 20 p.p.m., indicating the desirability of further testing with this compound.

Plasticizers

Several derivatives of New Crops fatty acids or oils have been tested as plasticizers for polyvinyl chloride and gave improved low temperature properties to the plasticized stock, such, for example, as lowering the brittle point to -43° C. to -51° C. as compared to -29° C. for the control, dioctyl phthalate. These new plasticizers include: The piperidide and the N,N-bis(2-ethoxyethyl)amide of mixed fatty acids from *Limnanthes douglasii* oil; the morpholide and N,N-bis(2-methoxyethyl)amide of the mixed fatty acids of parsley seed oil; and, in cooperation with NURDD, the morpholides and piperidides of erucic acid and of the mixed fatty acids of *Crambe abyssinica* oil.

The whole oil of *Cuphea llavea* was not compatible with polyvinyl chloride, but when mixed 1:1 with dioctyl phthalate, it proved to be an effective secondary plasticizer, lowering the brittle point to -39° C. It is anticipated that it may be possible to improve its effectiveness by modifying it, as, for example, by epoxidizing it.

Antimicrobial Activity of Some Fatty Acid Derivatives

All new materials prepared from New Crops oils or fatty acids are being submitted in cooperative research to Dr. A. F. Novak at Louisiana State University for screening against a wide range of microorganisms including gram positive and gram negative bacteria and pathogenic fungi.

Many of these materials have shown a wide range of activity. Since the list was compiled for the SURDD portion of the New Crops Research Report in January, 1963, the following additional materials have been prepared and submitted for screening:

6(7)-Acetamidostearic acid

6(7)-Acrylamidostearic acid

6(7)-Benzylamidostearic acid

6(7)-Propionamidostearic acid

Vinyl petroselinate

Adduct of petroselinic acid and hexachlorocyclopentadiene (HCCPD)

Adduct of methyl petroselinate and HCCPD

Adduct of ethyl petroselinate and HCCPD

Adduct of vinyl petroselinate and HCCPD

Petroselinylamine hydrochloride

"Duomeen" petroselinate hydrochloride

"Ethomeen" petroselinate hydrochloride

Oleylamine hydrochloride

"Duomeen" oleate hydrochloride

"Ethomeen" oleate hydrochloride

Lauraldehyde oxime

2-Decyl-2-hydroxymethyl-1,3-propanediol

2-Octyl-2-hydroxymethyl-1,3-propanediol

2-Heptyl-2-hydroxymethyl-1,3-propanediol

2-Hexyl-2-hydroxymethyl-1,3-propanediol

2-Chlorodecanoic acid

Methyl 2-chlorodecanoate

Neopentyl glycol ester of decanoic acid

2-Methoxydecanoic acid

n-Decyl thiocyanate

Future Research

Research is continuing on the development of utility for the oils, modified oils, and mixed component fatty acids from the seed of members of *Limnanthes*, *Umbelliferae*, and *Cuphea*. Since 5-eicosenoic acid can be separated in good yield from *Limnanthes*, and petroselinic acid from *Umbelliferae*, effort is currently also being expended to find utility for these acids and for chemicals derivable from them. The reactions being investigated with these unsaturated acids may be broadly grouped into three categories: Those which modify the double bond by introducing additional functional groups without cleavage; those which cleave the double bond; and those which leave the double bond unchanged but alter the compound in the vicinity of the carboxyl groups. Some of the modified products may be expected to have utility in the fields of plastics, plasticizers, protective coatings and biologically active compounds, and will be evaluated either in-house or by cooperating agencies.

Feeding tests with parsley seed meal and *Limnanthes douglasii* meal were reported in the SURDD portion of the New Crops Research Report for the January, 1963, meeting. It was stated that for the feeding of rats, parsley seed meal was comparable to soybean meal, but *L. douglasii* meal

was somewhat inferior, possibly because the presence of a glucosidic material (Ettlinger, M. G., and Lundeen, A. J., J. Am. Chem. Soc. 70, 1952 (1956), "The Mustard Oil of Limnanthes douglasii Seed, m-Methoxybenzyl Isothiocyanate.") might affect its nutritional value. Some research should probably be considered, therefore, to improve the nutritional performance of L. douglasii meal.

SURDD has recently been informed by Dr. Quentin Jones, NCRB (his memorandum to F. G. Dollear, SU, dated September 21, 1964), that, "We consider all species of this genus (referring to Cuphea) to be of poor crop potential and hence have discontinued procurement and crop evaluation of them." In view of this information, consideration should be given to a reduction in effort on utilization studies of Cuphea.

Summary

Limnanthes douglasii. Further work has been done on separation of the component fatty acids of oils of Limnanthes douglasii, resulting in a convenient separation of 5-eicosenoic acid from the remaining acids by fractional distillation of the mixed methyl esters. Though methyl 5,13-docosadienoate was also obtained in pure form by an involved fractional crystallization procedure and characterized, it does not seem feasible at present to separate the C-22 acids for utilization studies. Present plans are to concentrate on development of utility for 5-eicosenoic acid and derivatives, and for the mixed fatty acids and their derivatives. Arrangements are being made with the Alaska AES for the production of 1,000 pounds of Limnanthes douglasii seed for larger scale processing and utilization studies.

Petroselinic Acid. Since the possibility of growing fennel or other Umbelliferae which produce seed rich in petroselinic acid-bearing oil has continued to be encouraging, a study of the chemistry of petroselinic acid has continued. A variety of nitrogen-containing compounds and other derivatives have been prepared and submitted to various groups to be screened for possible utility. Many new compounds or mixtures of compounds have resulted from reactions which modify the carboxyl group, introduce additional functionality at the double bond, or cleave the double bond yielding fragments which are useful intermediates for further modification. It is expected that many of these new materials will have potential utility in the fields of plastics, plasticizers, protective coatings, biologically active compounds and other industrial and agricultural uses.

Cuphea. Though some species of Cuphea are known which produce seeds containing oil rich in the C_8 or C_{12} fatty acids, the predominant acid present in most Cuphea oils is decanoic acid. Numerous biologically active derivatives of decanoic acid have been prepared and screened in past utilization studies. A survey of the literature, discussed in the SURDD portion of the New Crops Research Report of January, 1963, revealed over 2,000 references to decanoic acid and described approximately 1,200 derivatives of decanoic acid, many of them having utility in soaps or other surface-active materials, plasticizers, pesticides, and many other uses. Thus, the utility of decanoic acid and other medium-chain length acids is well established if they can be produced at reasonable costs.

Present utilization studies for oils of Cuphea are being concentrated on finding utility for the oil itself. Studies are now in progress to evaluate the oil of Cuphea llavea as a plasticizer and in the preparation of alkyd resins.

Publications

1. Lida L. Placek. "A Review on Petroselinic Acid and Its Derivatives," J. Am. Oil Chemists' Soc., 40, 319-329 (August 1963).
2. Sara P. Fore, T. L. Ward, and F. G. Dollear. "The Preparation of Lauryl Alcohol and 6-Hydroxycaproic Acid from Petroselinic Acid," J. Am. Oil Chemists' Soc., 40, 30-33 (January, 1963).
3. Arthur F. Novak, Mary J. Fisher, Sara P. Fore, and Harold P. Dupuy. "Antimycotic Activity of Some Fatty Acid Derivatives," J. Am. Oil Chemists' Soc., 41, 503-505 (July 1964).

Patent Application

1. R. L. Holmes, J. P. Moreau, and A. F. Novak. "Certain Derivatives of Fatty Acids." Filed December 19, 1963, Serial No. 331,977.

Western Utilization Research and Development Division
Report for New Crops Meeting
Peoria, Illinois
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Seed and Oil Processing

Seed accessions.

In 1963 we obtained Dimorphotheca sinuata seed as follows: 230 pounds of mixed ray and disk seed from Ferry Morse Seed Company, Mountain View, California; 120 pounds of ray seed from Northrup, King and Company, Minneapolis, Minnesota; and 150 pounds of disk seed and 5 pounds of Osteopermum ecklonis seed from Crops Research Division, Beltsville, Maryland. Lesquerella gordonii seed (300 pounds) and L. fendleri seed (100 pounds) were furnished through H. S. Gentry from collections around Nogales, Arizona. L. densipila seed (41.5 pounds) was forwarded from the U. S. Plant Introduction Station, Glenn Dale, Maryland. D. sinuata seed (200 pounds) was ordered recently from Herbst Brothers. These, together with remainders of earlier samples, constitute our working stock.

Large-scale oil extraction from Lesquerella and Dimorphotheca seed.

One hundred thirty nine kg. of L. fendleri seed was processed in 30 kg. lots. Approximately 26.8 kg. of oil was obtained by extraction with reclaimed Skellysolve C. (b.p. 90-100° C.) representing 20.6% oil on the weight of seed corrected for 7% moisture. This oil was almost black in color and had an acid value of 14.6 rather than the usual 3 or 4. It contained a waxy solid (0.65% on seed weight). The color was not readily removed with clay or norite. It was determined that some of the Skellysolve C used was contaminated with water which may have accounted for the high acid value. Also, it appeared that the "wax" and the color were extracted into the oil because of the high boiling solvent (90-100° C.) used. Earlier extraction of a 5-kg. sample of this seed with Skellysolve B had produced a light red oil (21.8%) with an acid value of only 3.4. Later large-scale extractions (8, 10, and 30 kg. batches) of L. gordonii with Skellysolve F resulted in oil yields of 24.5-25.6%; acid values of 2.2-2.3; 64% lesquerolate by near infrared analysis. The extracted meal (moisture-free basis) showed:

Percent N	Percent Protein	Percent Crude Fat	Percent Crude Fiber	Percent Ash	Percent N.F.E.
5.55	34.7	1.12	13.5	7.57	43.11

We now have routinely adopted the procedure of using the lower-boiling hydrocarbon solvents and have instructed the operators of pilot-plant scale equipment to not use reclaimed barrels of solvent.

During this period we also extracted a 20-kg. batch of D. sinuata seed that had been dehulled and stored ca. 18 months at 34° F. The acid value of the oil had increased to about 14 from the normal 4-5 during storage. To fill our needs for low acid value dimorphotheca oil, 41.5 kg. of the ray and disk seed were dehulled in 51% yield and extracted leading to 23.6% oil (dehulled seed basis) with acid value 8.5. A 17.9-kg. sample of L. densipila seed was cleaned. The cleaned seed recovered weighed 11.7 kg. (65.5%). A sample (6.9% moisture) contained 29.2% oil on the dry seed weight. The

olive-green oil had an acid value of 3.0, refractive index = 1.4789 and the NIR spectrum of a 100 λ/10 ml. $CHCl_3$ solution (1-cm.) had two peaks, d_{2760} = 0.304 and d_{2790} = 0.315. The extracted meal was analyzed with the following results, expressed on a moisture-free basis:

Percent N	Percent Protein	Percent Crude Fat	Percent Crude Fiber	Percent Ash	Percent N.F.E.
3.42	21.3	1.22	10.8	7.75	58.93

Additional physical constants and analytical results obtained on oils and meals were:

		$\alpha_D^{25.5°\ C.}$	25°C. ρg./ml.
Dimorphotheca oil	$N_D^{25.0}$ = 1.4916	+ 6.62°	0.9534
Lesquerella oil		+ 2.18	0.9297

	Percent N K.W.G.	Percent Protein (N x 6.25)	Percent Crude Fat	Percent Crude Fiber	Percent Ash	Percent N.F.E.
Dimorphotheca meal	9.39	58.69	2.87	4.60	8.46	25.4
Lesquerella meal	5.49	34.31	1.24	11.8	6.97	45.7

Extraction of fatty acids from high acid value oils.

Since our previous experience had shown that alkali refining was very tedious with the hydroxy acid oils, we used extraction with aqueous-methanol (as suggested by Prof. A. Rheineck) to reduce the acid values. Four extractions of 25 kg. of lesquerella oil (acid value 14.6) with equal volumes of 95% methanol reduced its acid value to about 5. The fatty acid concentrate had an acid value of 61. A similar treatment of 5 kg. of dimorphetheca oil (acid value 14) with 10% H_2O-90% methanol lowered the acid value of the oil (84%)to 5.4 and gave an acid concentrate (16%) of acid value 44. The oils and fatty acid concentrates obtained were utilized as starting materials for ester and acid preparation.

Chemical Studies

Preparation and purification of esters.

The ion-exchange procedure was investigated with lesquerella oil. For example, 120 grams of lesquerella oil in a 3 benzene:4 oil:20 methanol solution was percolated through a column containing 90 cc. of Amberlyst XN-1001 ion-exchange resin in the methoxide form at a flow rate of 0.33 ml./minute (residence time 2.25 hours). The column was washed with 600 ml. of methanol at the same flow rate. According to glycerol recovery, the reaction was nearly 100% complete. Fractional distillation (10-inch Vigreaus; b.p. 200-213°/0.9 mm.) of 109.4 grams of the esters yielded 43.05 grams (39.4%) of esters having d_{2760} = 0.344 to 0.363 (89-94% purity). With the large lot 25 kg.) of lesquerella oil base-catalyzed alcoholysis was employed. After the usual treatment, the esters were distilled in a

Consolidated Brush Still at 150-180° C./5-10 μ Hg in 1500-gram lots. Methyl lesquerolate (86-88% purity) recoveries of 29-32% were realized. L. densipila oil was converted to esters by base-catalyzed alcoholysis and distilled in the Brush Still. Fractions (165-175° C., 5 μ Hg) containing 2 hydroxy acid esters were obtained. These had GLC retention times identical to methyl ricinoleate and densipolate on PEGS. The authentic methyl densipolate was prepared from a densipolic acid concentrate obtained from Lesquerella densipila seed oil. Chromatography on silicic acid yielded 40% light yellow nonhydroxy acids and 60% green hydroxy acids (lit. value 50%). A concentrate of methyl densipolate was prepared by fractionating mixed acids with urea and esterifying the noncomplexing acids. The green esters were decolorized on a 2-inch column of alumina. The main problem in isolating methyl densipolate from mixed esters of Lesquerella densipila seed oil is the separation of densipolate which has two double bonds from another hydroxy ester which has only one. When the method of Emken, Scholfield and Dutton for use of the silver ion-exchange resin column was followed, the mono-olefinic ester was eluted with methanol while the cis, cis dienoate complexed strongly and did not elute. By consideration of possible interactions with silver ion we found densipolate could be easily displaced by gradually changing the solvent to acetonitrile. Subsequent chromatography on silicic acid eliminated traces of linoleate and colored matter introduced from the resin. Pure methyl densipolate (TLC, GLC) has major infrared peaks (neat) 2.9 μ (OH), 3.4 μ (CH), 5.75 μ (C=O), 6.9 μ (CH doublet), 8.35 μ 8.5 μ (O-CH_3 doublet), 9.4 μ and 9.8 μ and ultraviolet absorption λ_{max} 185.0 ϵ_{max} 25,700, calculated 73.5, H 11.0; found C 73.4, H 10.9. An interesting sidelight was the recovery of a crystalline compound from the high boiling fractions of the mixed L. densipila esters. A sample (recrystallized from methanol) of m.p. 134-136° C. has an NMR spectrum similar to but not identical with β-sitosterol. It is attractive to speculate that the "wax" obtained in seemingly copious quantities from the L. fendleri oil may be largely steroidal in nature.

Large-scale preparations of methyl dimorphecolate were carried out by base-catalyzed alcoholysis followed by an improved solvent-solvent partition method. As much as 650 grams of mixed esters as a 10% solution in 10% aqueous methanol were extracted six times with 650 ml. portions of Skellysolve F leading to 40-45% yields of methyl dimorphecolate of 90-92% purity. These esters were suitable for use in our other studies.

Acetylation of methyl lesquerolate.

Thirty-four grams of distilled methyl lesquerolate was acetylated with 40 ml. of acetic anhydride in 120 ml. pyridine at 100° C. for 2 hours. The product was fractionally distilled. The main fraction distilled at about 200° C./250 μ Hg had d_{2760} = 0.023; d_{230} = 0.026; N_D^{25} = 1.4541; and $[\alpha]_D^{25}$ + 20.63 (neat).

Preparation of Dimorphotheca and Lesquerella acids.

The 5 kg. of Lesquerella fatty acid concentrate (acid value 53) recovered from the high acid value oil was saponified, acidified, washed, and dried to yield 4.2 kg. of acids (acid value 179). Similar procedures will be carried out on the 800 grams of fatty acid concentrate (acid value 44) from the Dimorphotheca oil. These acid preparations are of value for use in the alkali fusion and coatings studies.

Preparation and characterization of hydroxy acid lactones.

In a model study authentic, liquid 12-stearolactone (infrared, NMR and mass spectral evidence) was prepared in 8% yield by published methods. A dimeric lactide (m.p. 62-63°) (molecular weight: calculated 565; found 526, 532) was obtained in 11% yield and 15% unsaturated acid was recovered. Both the lactone and the lactide were saponified back to authentic 12-hydroxystearic acid. We have concluded that our earlier report of the apparent formation of a solid lactone (m.p. 35.5-37.5° C.) during phosphorus ester preparations with model hydroxy acids from castor oil was in error. The material found there evidently was methyl stearate (lit. m.p. 38° C.); an impurity in the starting 12-hydroxystearate which was concentrated during the purification steps.

Dehydration of methyl lesquerolate.

Studies on the dehydration of castor, dimorphotheca and lesquerella oils reported in the last period were extended by studies on methyl lesquerolate with methyl ricinoleate as a reference compound. Methyl ricinoleate heated 15 minutes in the presence of 1% $NaHSO_4$ at 212-240° C./20 mm. Hg showed a decrease in infrared hydroxyl of 71% and an increase in conjugated diene. The 15-minute heating time was less effective with methyl lesquerolate (49% reduction in infrared hydroxyl) so the product was reheated with fresh catalyst for 30 additional minutes (85% hydroxyl reduction).

Additional work in this area is described in the Contract Research section of WU's report.

Pyrolysis of methyl lesquerolate.

Previous preliminary studies on methyl ricinoleate were extended by evaluation of variables affecting yields and purities, e.g., feed rate, pyrolysis temperature, and reactant introduction mode. The improved techniques were then applied to methyl lesquerolate pyrolyses. In a typical experiment distilled methyl lesquerolate was fed at constant rate (1.42 ml./minute) by a gear pump through a capillary extending beneath the surface of a lead alloy bath. Product yield was 1.25 grams/minute at 575-578° C. which contained 69% methyl tridecenoate (near infrared at 1.65 μ). The product was fractionally distilled and served as a starting material for the 13-amino tridecanoic acid synthesis.

To aid in evaluating these studies, quantitative GLC was attempted. Detector responses were determined for starting materials and products relative to methyl palmitate. Known amounts of methyl palmitate were added to pyrolyzates and GLC's were run. Unknowns were assumed to have methyl palmitate's response. A sample of pyrolyzed methyl ricinoleate gave these results: (Components are listed in order of elution).

	Trial I	Trial II
Unknowns	3.5%	3.1%
Heptaldehyde	23.2%	22.4%
Unknown	2.2%	3.9%
Methyl undecenoate	39.6%	39.0%
Methyl palmitate, 25.9% added	28.3% found	28.0 found
Unknowns	4.8%	4.1%
Methyl ricinoleate	26.6%	27.4%

Ketostearate, which might have been formed by isomerization, would not be distinguishable from ricinoleate under the GLC conditions used. Similar analyses on four pyrolyzates of methyl lesquerolate showed as many as 34 peaks. A typical result follows:

	Weight, %
C_0-C_7	6.8
Heptaldehyde	32.5
C_7-C_{13}	5.0
Methyl tridecenoate	40.4
C_{13}-C_{16}	0.3
C_{16}-C_{20}	2.8
Methyl lesquerolate	11.7
C_{20}-C_{22}	0.5
Methyl palmitate	28.1 (29.1 added)

The array of products obtained points up the complexity of the pyrolysis reaction. The appearance of unsaturated acids of less than C_{13} chain length is in accord with the occurrence of known secondary reactions and will be useful in judging the effectiveness of reaction modifications designed to suppress such reactions.

Preparation of 13-amino methyl tridecanoate from methyl tridecenoate.

To prepare the monomer for nylon-13 polymer, distilled methyl tridecenoate was hydrobrominated by bubbling a 15% excess of anhydrous HBr through an ice-cooled Skellysolve B solution of the ester under ultraviolet irradiation while dropping in benzoyl peroxide in benzene to induce bromination of the terminal carbon atom (method of R. G. Jones, J.A.C.S. 69, 2350-2354). Crude product (96%) was obtained. NMR indicated only ω-bromination and TLC showed one component. The crude bromo-ester was treated in methanol with a 20-fold

excess of anhydrous ammonia and the reaction course was monitored by TLC. After 72 hours at room temperature, infrared evidence suggested that both amino ester and amide were formed. The reaction was terminated after six days and the product isolated. The product was obviously a mixture (possibly containing some polymer) and was difficult to free from inorganic salts. Best results were obtained via alkaline hydrolysis followed by acid hydrolysis and ion-exchange treatments leading to 13-amino tridecanoic acid m.p. 159-161° (lit. value 163°) with the expected infrared spectrum after recrystallization from methanol. We are now engaged in purifying larger quantities of this amino acid which will be polymerized to the nylon-13 polyamide.

Alkaline fusion of lesquerolic and dimorphecolic acids.

Alkaline fusion of lesquerolic acid has been reported to lead to dodecanedioic acid (24% yield 90% purity) and octanol-2 (M. O. Bagby, J. Org. Chem. 26, 4735 (1961) and C. R. Smith, Jr., M. O. Bagby and I. A. Wolff, U.S. Patent No. 3,057,893; October 9, 1962). Similar treatment of castor oil is well known. In model studies employing sodium ricinoleate (35-40% in water at 90° C.), excess 85% aqueous sodium hydroxide, and 5% lead oxide as suggested by the Baker Castor Oil Company, we obtained 70% sebacic acid at 250° C. and 69% at 265° C. When the sodium salt of lesquerolic acid (water-insoluble) was added in a 95% ethanol solution to the caustic bath at 270-320°, a 62% yield of crude dodecanedioic acid, m.p. 110-122° C., was obtained. A 48% yield of once-recrystallized product (ethyl acetate) had m.p. 125-128° C. (lit. m.p. 129° C.). Use of the more soluble potassium lesquerolate in hot aqueous solution led to a 46% yield of product, m.p. 126-129° C. At this time we became aware of the availability of cyclo-dodecene (derived from butadiene trimer) which supposedly would lead to dodecandioic acid at a materials' cost of 61 cents/pound. Since we could not hope to compete with this price except with the most optimistic estimates of eventual oil cost and synthetic results, we abandoned further attempts at improving dibasic acid yields and went on to ω-hydroxy acid preparation.

Relatively low temperature (180-200°) alkaline fusion of ricinoleic acid has been reported to yield octanone-2 and 36% of ω-hydroxy acids. Initial trials at 185-200° C. using sodium ricinoleate or lesquerolate led to very low yields (12% and 4%, respectively) of the ω-hydroxy acids and considerable amounts (21% and 40%, respectively) of the dibasic acids. Analysis of the crude mixtures was accomplished by acid-catalyzed esterification followed by GLC and TLC. Consideration of the proposed mechanisms operating in such systems led us to carry out the reactions in the presence of excess 2-octanol. In companion experiments the effect of added octanol was striking. In a system swept by nitrogen which removed octanol, the following results were noted by GLC analysis of the crude esterified product: dibasic acid (40%), ω-hydroxy acid (39%), starting acid (14%), and unknown (7%). With about a 4:1 mole ratio of octanol:lesquerolic acid (octanol retained by refluxing), the following results were noted: dibasic acid (14%), ω-hydroxy acid (62%), starting acid (8%), and unknowns (16%). The latter result has

been obtained repeatedly. Amounts of products from six different runs analyzed were: dibasic acid (12-21%), ω-hydroxy acid (59-66%), starting acid (5-13%), and unknowns (6-12%). Thus, we have found in preliminary trials that considerably improved yields of ω-hydroxy acids can be obtained by low-temperature alkaline fusion in the presence of excess 2-octanol. These results also lend some support to the mechanisms of such reactions proposed in the literature (cf., J. Chem. Soc. 753 (1947); Tetrahedron 8, 246 (1960)). The results also suggest that considerable control of the types and amounts of reaction products may be achieved in such reactions by the addition of suitable reactants. Patent disclosures have been made.

Recently we have completed preliminary studies on the alkaline fusion of dimorphecolic acid. Cleavage in the absence of 2-octanol at 200° and 265° yields essentially the same mixture as assessed by GLC. The esterified acid mixture contains hydroxydecanoic acid(s) (50%) and three higher molecular weight compounds (50%) in a 2:3:1 ratio. No absorption at 231 mμ for dimorphecolate was observed in the ultraviolet. In the presence of excess 2-octanol at 180° the fusion of dimorphecolic acid led to essentially quantitative formation of hydroxydecanoic acid(s) as assessed by GLC of the esterified product. Column chromatography on silicic acid (ether-Skelly F) gave a 20% yield of a single hydroxy acid methyl ester (GLC and TLC). NMR, infrared and mass spectral data support the structure: methyl 9-hydroxydecanoate. Saponification and acidification led to 9-hydroxydecanoic acid, m.p. 32-38°. The primary volatile product (mass spectral data) appears to be 1-hexanol. Thus, in contrast to the alkaline cleavage of the β-hydroxyene systems of ricinoleic and lesquerolic acids which yield primary alcohols, the cleavage of the vicinal dienol of dimorphecolic acid leads to a secondary alcohol. This reaction and the products therefrom are currently being examined more completely. The hydroxy acids obtained in these studies will be utilized in the preparation of linear polyesters possibly under our polymerization contract.

Preparation of vinyl esters for polymerization studies.

Vinyl 9- and 12-hydroxystearate and vinyl 14-hydroxyeicosanoate were prepared and purified. Methyl dimorphecolate and lesquerolate were reduced to saturated hydroxy acid esters, hydrolyzed and the free acids reacted with vinyl acetate by slight modifications of the published method (cf., J. Polymer Sci. 1, 2067 (1963)). Vinyl 9-hydroxystearate has m.p. 53.5-54.8° C.; vinyl 14-hydroxyeicosanoate has m.p. 63.5-65.0° C. Preparation and purification systems also were worked out for the synthesis of vinyl 9- and 12-chlorostearate and vinyl 14-chloroeicosanoate. The vinyl hydroxy derivatives were treated with thionyl chloride and pyridine in boiling carbon tetrachloride, washed with water, carbonate solution, and water, and decolorized through norite and slumina. The yellow, fluid oils obtained in about 90% yield showed single spots on chromatostrips and had no detectable hydroxyl or trans olefin bands in the infrared.

Considerable effort was expended in attempts towards preparing acylated vinyl esters of the hydroxy acids. Acetylation followed by vinylation

resulted in excessive transesterification and inseparable mixtures. The most successful acetylation procedure involved treatment of the vinyl hydroxy ester with two equivalents of acetyl chloride in cold carbon tetrachloride followed by the addition of two equivalents of pyridine. The system was heated (100°, 2 hr.) and worked up to yield a product (80-90%) showing one spot on TLC and having an acceptable infrared spectrum. Extension of this technique to caprylyl chloride was unsuccessful.

Urethane foams.

Rigid foams were prepared from a dimorphetheca oil-triisopropanolamine polyol containing small amounts of linseed oil (boiled). It was hoped that the linseed oil might promote oxidative crosslinking of the foam polymer and, thus, that the strength of the foam would increase on aging. There seems to be a small increasing in compressive strength at the end of the two months. The test will be continued to see if this increase is real.

Days Aged	Compressive Strength (Density = 2.0 pounds/foot^3)	
	2 Percent Linseed Oil* p.s.i.	4 Percent Linseed Oil* p.s.i.
14	30	28
54	37	34

* In foam polymer containing 16% dimorphotheca oil.

Analytical Studies

Far ultraviolet analysis of unsaturated fatty acids.

This area of the ultraviolet spectrum (175-200 mμ) has only recently become routinely useable through the availability of nitrogen-purged commercial spectrophotometers. The classical work on fatty acid derivatives was done many years ago by Rusoff, et al., (J. Am. Chem. Soc. 67, 673 (1945)) using vacuum equipment and photographic recording methods, and certain of their results were not clearly understandable. We began the study on this area of the ultraviolet spectrum with an aim to assessing the effect of increasing the number of methylene interrupted double bonds, the effect of isomerizing cis double bonds to trans, and the effect of substituents near the double bond. All this we hoped would lead to development of another useful analytical tool for fatty acid chemistry. The methods of measuring the spectra are essentially those described in earlier publications from our laboratories (Tetrahedron Letters 16, 560 (1961); J. Org. Chem. 27, 345 (1962)). Compounds of the best purity obtainable were used e.g., GLC

standards from Hormel and NIH, samples generously shared by our colleagues at the other Regional Labs, and many of our own preparations. The results of these studies are shown in Table I.

Table I

Spectral Data for Pure Compounds

Methyl Ester	λ_{max} (mμ)	ϵ	$\int_{\lambda_{max}}^{\lambda_{min.}} \epsilon \, d\bar{\nu}$ cm./mole x 10^8	f	$\int_{180}^{\lambda_{min.}} \epsilon \, d\lambda$ cm.^{3}/mole
cis-9-hexadecenoate	184.0	12900	442	0.38	21.1
cis-6-octadecenoate	184.0	13000	452	0.39	21.2
cis-9-octadecenoate	184.0	13000	441	0.38	21.8
cis-11-octadecenoate	184.0	13150	458	0.40	22.2
cis-12-octadecenoate	184.0	13100	453	0.39	21.8
cis-11-eicosenoate	184.0	13150	452	0.39	21.5
cis-13-docosenoate	184.0	12850	449	0.39	21.3
cis-15-tetracosenoate	184.5	12950	445	0.38	21.8
12-hydroxy-cis-9-octadecenoate	184.0	13800	476	0.41	23.2
14-hydroxy-cis-11-eicosenoate	184.5	13700	460	0.40	23.1
cis-12,13-epoxy-cis-9-octadecenoate	184.0	13900	453	0.39	21.8
12-methoxy-cis-9-octadecenoate	185.0	12800	439	0.38	22.4
12-methoxy-trans-9-octadecenoate	189.0	11200	342	0.29	22.4
trans-9-octadecenoate	187.0	11900	372	0.32	21.8
12-hydroxy-trans-9-octadecenoate	186.5	11450	383	0.33	21.4
cis-9-cis-12-octadecadienoate	190.0	18750	687		44.2
cis-5-cis-13-docosadienoate	184.5	25750	873		43.1
12-hydroxy-cis-9-cis-15-octadecadienoate	185.0	25700	850		43.8
trans-9-trans-12-octadecadienoate	192.0	16050	590		41.7
all cis-9,12,15-octadecatrienoate	192.5	25150	888		65.3
all cis-5,8,11,14-eicosatetraenoate	193.5	31800	1193		89.1

Table I Continued

Methyl Ester	λ_{max} (mμ)	ϵ	$\int_{\lambda_{max}}^{\lambda_{min.}} \epsilon \, d\nu$ cm./mole x 10^8	f	$\int_{180}^{\lambda_{min.}} \epsilon \, d\lambda$ cm.^{3}/mole
all cis,5,8,11,14,17-eicosapentaenoate	193.5	37400[a]	1439		106.8
all cis,4,7,10,13,16,19-docosahexaenoate	194.0	43600[a]	1690		130.8
ω-(2-n-octyl-cyclopropenyl) octanoate	ϵ_{195}	5440			20.4
12-oxo-cis-9-octadecenoate	ϵ_{183}	10900			22.4
12-oxooctadecanoate	ϵ_{183}	940			1.3
cis-9,10-epoxyoctadecanoate	ϵ_{184}	315	4.5[b]		0.6
12-methoxyoctadecanoate	ϵ_{185}	1026	18[c]		
	ϵ_{189}	493	10[d]		1.4
12-hydroxyoctadecanoate	ϵ_{184}	430	11.5[b]		0.8
octadecanoate	ϵ_{184}	110			0.2
cis-2-octene	181.5	13450	438	0.38	
trans-2-octene	184.0	10450	350	0.30	

[a] Average of three determinations.

[b] 184 mμ - $\lambda_{min.}$

[c] 185 mμ - $\lambda_{min.}$

[d] 189 mμ - $\lambda_{min.}$

These data may be summarized by noting: ϵ_{max} for cis-monoenes is 13,000 ± 150; ϵ_{max} for methylene-interrupted olefins increases linearly but is not an even multiple of ϵ = 13,000 for oleate; the integrated area under the absorption curve with wavelength in mμ (last column, Table I) is a good quantitative measure of total unsaturation and increases in an integral fashion as does the number of double bonds; and the more theoretically meaningful integrated intensity with wavelength in cm^{-1} (fourth column, Table I) can be used to calculate oscillator strengths which are in good agreement with the value predicted by the free-electron approximation. Also, as found by some other workers, trans double bonds absorb at longer wavelengths with lower intensities than do cis double bonds. We re-examined the absorption of cis and trans 2-octenes to clear up an apparent error in the literature that people have

explained away rather than check for years. The original claim (J. Chem. Phys. 17, 466 (1949)) showing a reversal of cis and trans wavelength positions and absorption intensities for cis and trans 2-octene seems in error since all other examples of this type in the literature and our recheck of these specific compounds (cf. Table I) correlate well. These data, experimental procedures, and more detailed discussions have been combined in a manuscript being prepared for the Journal of Organic Chemistry.

Contract Research

Polymerization studies.

Work at the University of Arizona sponsored by all four laboratories during this period included studies on vinyl esters derived from Lesquerella and Dimorphotheca. Homopolymers and vinyl acetate- and vinyl chloride-copolymers were prepared from the vinyl esters of 9-chlorostearic acid, 12-chlorostearic acid, 14-chloroeicosanoic acid, 9-hydroxystearic acid, and 14-hydroxyeicosanoic acid. This study was a continuation of earlier work and was designed to assess the effect of substituent position on polymer properties. Homopolymers were prepared in benzene solution at 65° employing 2,2'-azobisisobutyronitrile as initiator and in aqueous emulsion systems using Triton X-301 and potassium persulfate at 60° C. Copolymers with vinyl acetate were prepared in benzene solution as above and with vinyl chloride in an emulsion system. The results are presented in the Tables. The polymer samples have been forwarded to WU and certain ones will be subjected to further evaluation.

Surface coatings from Lesquerella and Dimorphotheca oils.

The contract covering this work was activated February 8, 1963, and the contractor has made good progress in preparing and evaluating surface coatings from lesquerella and dimorphotheca oils and from castor oil as a reference oil in some instances. Varnishes prepared from dimorphotheca oil-diisocyanate adducts modified with various polyols were found to have excellent properties. Tough, hard, flexible films with fast dry times (as low as 30 minutes with 0.05% Co) and good water and alkali resistance have been obtained. Isocyanates investigated have included toluene diisocyanate (TDI), polymethylenepolyphenyl isocyanate (PAPI), 3,3'-dimethyl 4,4'-biphenylene diisocyanate (TODI), methylene bis-4-phenyl isocyanate (MDI), its saturated analog ($H_{12}MDI$), metaphenylene diisocyanate, safflower isocyanate, and ethyl isocyanate. Polyols used included linseed monoglyceride, trimethylolpropane, various rosin pentaerythritol esters, bisphenol A, and hydrogenated bisphenol A. Several more promising varnishes were subjected to weathering tests. Results after 110 days showed that the dimorphotheca-TDI-rosin pentaerythritol diester or trimethylolpropane combinations showed no failures and their color was less than a commercial polyurethane varnish used as a control. One varnish prepared with a commercial phosphorous-containing diol showed intumescence superior to commercial alkyd or polyurethane varnishes in standard fire cabinet tests.

Table II

Solution and Emulsion Homopolymerization of the Vinyl Esters

Vinyl Ester (polymerization system) Sample Number	Conversion %	Inherent Viscosity[b]	Softening Range °C	Reprecipitation		Elemental Analysis			
				Solvent	Non-solvent		C	H	Cl
Vinyl 9-chlorostearate						Calcd.	69.63	10.81	10.28
I-148-1 (solution)	36	0.101[c]	25	benzene	methanol	Found	69.58	10.93	9.85
I-149-15 (emulsion)	25	0.155		THF	water	Found	70.48	8.83	9.42
Vinyl 12-chlorostearate						Calcd.	69.63	10.81	10.28
I-148-2 (solution)	60	0.142	25	THF	methanol	Found	70.13	10.69	10.05
I-149-16 (emulsion)	40	0.157	25	THF	water	Found	70.12	10.93	10.05
Vinyl 14-chloroeicosanoate						Calcd.	70.88	11.08	9.50
I-148-4 (solution)	20	0.112[c]	25	benzene	methanol	Found	70.95	11.56	9.30[d]
Vinyl 9-hydroxystearate						Calcd.	73.57	11.73	
I-148-3 (solution)	65	0.127	70-2	THF	methanol	Found	71.12	11.85	
I-149-17 (emulsion)	51	0.412	69-70	THF	water	Found	73.60	13.36	
Vinyl 14-hydroxyeicosanoate						Calcd.	74.52	11.94	
I-148-5 (solution)	36	0.155	78-80	THF	methanol	Found	74.05	11.92	
I-149-18 (emulsion)	65	0.232	79-82	THF	water	Found	73.52	11.66	

[a] Solution polymerizations were carried out in a Fisher "Isotemp" oven at 65° C. and the emulsion polymerizations were tumbled in a constant temperature bath at 65° C.

[b] Inherent viscosity of a tetrahydrofuran solution (0.4 g. of polymer/100 ml. of solvent) measured at 30° C. in a Number 50 Cannon-Fenske viscometer.

[c] Measured in a benzene solution (0.6 to 0.8 g. of polymer/100 ml. of solvent).

[d] Elemental analysis corrected for residue: C, 61.04; H, 9.95; Cl, 8.00; residue, 13.97.

Table III

Solution Copolymerization of the Vinyl Esters with Vinyl Acetate (Molar Ratio 1:9)[a]

Vinyl Ester[b] (Sample Number)	Conversion[c] %	Inherent Viscosity[d]	Softening Range °C	Reaction Time (hr.)	Analysis Calcd. C	Calcd. H	Calcd. Cl	Found C	Found H	Found Cl
Vinyl 9-chlorostearate (I-148-6)	36	1.47	< 25°	63	60.07	8.19	3.16	59.40	8.24	3.32
Vinyl 12-chlorostearate (I-148-9)	55	0.362	< 25°	30	60.07	8.19	3.16	61.00	8.60	3.86
Vinyl 9-hydroxystearate (I-148-10)	74	0.38	< 25°	63	61.07	8.42		61.73	8.68	
Vinyl 14-chloroeicosanoate (I-149-13)	54	0.318	< 25°	40.5	60.64	8.42	3.09	61.84	8.73	3.68
Vinyl 14-hydroxyeicosanoate (I-149-14)	82	0.328	< 25°	63	61.63	8.65		61.58	8.70	

[a] At 65° C. in a Fisher "Isotemp" oven.

[b] Each polymerization batch contained about 5 g. of monomers (0.393 mole VAC and 0.00435 mole vinyl ester).

[c] Conversion measured after four reprecipitations from a tetrahydrofuran solution into 50:50 water:methanol.

[d] Inherent viscosity of a tetrahydrufuran solution (0.4 g. of polymer/100 ml. of solvent) measured at 30° C. in a Number 50 Cannon-Fenske viscometer.

Table IV

Emulsion Copolymerization of the Vinyl Esters with Vinyl Chloride[a]

Vinyl Ester (polymerization batch)	Weight Ester g.	Weight VCl g.	Conversion[b] %	M.P. °C	Inherent Viscosity	Elemental Analysis C	H	Cl	VCl/V_E[d]
Vinyl 9-chlorostearate									
92-1	2.0	8.0	69	160-180	1.21				
92-2	3.0	7.0	63	155-170	1.11				
Vinyl 14-chlorostearate									
92-3	2.0	8.0	52	~145	0.72				
92-4	3.0	7.0	45	~145	0.69				
Vinyl 9-hydroxystearate									
90-1	2.0	8.0	65	145-165	0.79	45.47	6.16	44.87	19.6/1
90-2	2.5	7.5	68	180-200	0.83	47.29	6.48	42.48	15.5/1
90-3	3.0	7.0	54	185-195	0.87	47.31	6.47	42.66	15.8/1
Vinyl 14-hydroxyeicosanoate									
90-7	2.0	8.0	63	165-175	0.80	44.30	5.83	46.55	25.8/1
90-8	2.5	7.5	65	170-185	1.60	44.35	5.96	46.65	26.1/1
90-9	3.0	7.0	55	183-210	0.87	46.19	6.83	44.42	19.5/1

[a] The polymerization batches were tumbled in a constant temperature bath at 60° C. for 72 hours.

[b] Conversion after nine reprecipitations from tetrahydrofuran into water and three reprecipitations into methanol.

[c] Inherent viscosity of a tetrahydrofuran solution (0.4 g. of polymer/100 ml. of solvent) measured at 30° C. in a Number 50 Cannon-Fenske viscometer.

[d] Molar ratio of monomer (vinyl chloride/vinyl ester) calculated on basis of chlorine analysis.

The very slow dry time of raw dimorphotheca oil in the presence of 0.05% cobalt (30 hours) with heavy wrinkling can be strikingly improved by added linseed oil or t-butyl peroxide. Ten percent raw linseed drops the drying time to 3 hours and the film is hard and smooth. Tert-butyl hydroperoxide (0.1%) lowers the time to 7 hours; the film is smooth and hard, but shows syneresis in 10 days. In a more detailed examination of the effect of linseed oil, a linear relationship was noted between amount of linseed oil added (2-10%) and the tack-free time (70-20 hours). The tack (0-500 grams), however, increases linearly over the same linseed range. None of these films wrinkled. The contractor discussed these results with respect to the relative lability of the cis and trans isomers to air oxidation and polymerization.

Considerable time was spent in investigating the dehydration of castor oil as a model for work with lesquerella oil. Dehydration catalysts studied included sulfuric acid, sodium bisulphate, phosphoric acid, boric acid, tungstic anhydride, tungstic acid, alumina, and activated clays (Filtrols). Reactions were followed by viscosity measurements at 15-minute intervals and the final products were analyzed by infrared, ultraviolet and hydroxyl value. Films were cast (0.05% Co drier) and drying times checked. Appreciable dehydration occurred with sodium bisulphate and sulfuric or tungstic acids. The latter catalyst gave greatest yields of conjugated diene and lowest hydroxyl value, and the proportion of trans-trans isomer is higher. The drying time of a film (0.05% Co) of this material was 1.5 hours, but the Zapon tack was greater than in the less-dehydrated samples. It was suggested that this effect was related to the relative amounts of the various conjugated isomers. Work directed towards exploring this relationship is still in progress.

Lesquerella oil was dehydrated in the presence of sulfuric or tungstic acids and the products analyzed as described above. The drying times were 2 hours, but as would be expected, the films were softer and tackier than corresponding dehydrated castor oil films.

The results thus far demonstrate that dimorphotheca oil should have a place in specialty coatings. The data on drying time and its relationship to the various unsaturated isomers may well provide clues to the design of better air-dried coatings based on either dimorphotheca oil or dehydrated castor or lesquerella oils.

Presentations, Publications, and Patents

Preparation of Phosphorous Esters of Long-Chain Hydroxy Fatty Acids.
M. J. Diamond, T. H. Applewhite, R. E. Knowles, and L. A. Goldblatt.
Presented at the 36th Fall Meeting of the American Oil Chemists' Society, Toronto, Canada, October 1-4, 1962.
J. Am. Oil Chemists' Soc. 41, 9-13 (1964).

A Study of Film Forming Characteristics of Dimorphotheca Oil.
A. E. Rheineck and H. Sobal.
Official Digest 34, 862-883 (1962). Published as a result of a cooperative study using dimorphotheca oil provided by WU.

Chromatographic Analysis of Seed Oils. II. Fatty Acid Composition of Dimorphotheca Oil.
R. G. Binder, T. H. Applewhite, M. J. Diamond, and L. A. Goldblatt.
J. Am. Oil Chemists' Soc. 41, 108-111 (1964).

Preparation of Heptaldehyde and Tridecylenic Acid.
L. A. Goldblatt and R. E. Knowles.
U. S. Patent application filed August 26, 1963.

Hydroxy Unsaturated Oils. I. Processing Dimorphotheca and Lesquerella Seed for Oil and Meal Recovery.
R. E. Knowles, K. W. Taylor, G. O. Kohler, and L. A. Goldblatt.
J. Agr. and Food Chem. 12, 390-392 (1964).

Hydroxy Unsaturated Oils. II. Preparation and Characterization of Methyl Dimorphecolate and Methyl Lesquerolate from Dimorphotheca and Lesquerella Oils.
M. J. Diamond, R. G. Binder, R. E. Knowles, and L. A. Goldblatt.
J. Am. Oil Chemists' Soc. 41, 430-433 (1964).

Oilseed Composition of Two Species of Dimorphotheca Grown at Five Locations in the U. S.
R. E. Knowles, L. A. Goldblatt, G. O. Kohler, S. J. Toy, and J. R. Haun.
Accepted for publication in Economic Botany.

Dehydration of Lesquerolates.
L. A. Goldblatt and R. E. Knowles.
U. S. Patent application filed October 2, 1963.

SEED MEAL SCREENING FOR AMINO ACID COMPOSITION

This program was continued on a limited scale by analysis of 21 species from 11 families. Of these, 10 species were high in methionine ranging from 2.0 to 3.5 g./16 g.N.

To date the seed from 370 species have been analyzed for their amino acid composition. Of these the results from 200 species were evaluated and interpreted with the aid of statistics. Lysine, methionine, arginine, proline, hydroxyproline, and glutamic acid showed the greatest variation. The amino acid compositions showed overall similarities, but there were significant differences between the contents of certain amino acids in selected plant families. For example the lysine content of the Cruciferae and Leguminosae (mean 5.0 and 5.2 g./16 g.N. respectively) tended to be high and that of the Compositae low (mean 3.5 g./16 g.N.). Methionine content of the Compositae tended to be high (1.8 g./16 g.N.) of the Cruciferae average (1.5 g./16 g.N.) and of the Leguminosae low (1.1 g./16 g.N.).

The percent of total nitrogen found as amino acid amine nitrogen ranged from 52 to 92%. Those seed meals containing large amounts of nitrogen unidentified as amino acid are of interest because the analyses indicate the seed contains unknown nitrogen-containing compounds in large amounts. In Table 1 are listed 26 seed meals containing large amounts of unidentified nitrogen. In some cases the seed meals also contain one or more unidentified substances detected as elution peaks in the analysis. Much of the ammonia reported is related to aspartic and glutamic acids but some probably arise from the decomposition of unknown components. In still others no major unknowns were detected on the chromatograms and the ammonia nitrogen was low indicating the presence of nitrogen-containing compounds not detected in the ion-exchange separation as carried out. These seed meals are of interest for further investigation in order to determine the nature of the nitrogen-containing substances in the seed that were not identified.

Table 1. Seed Meals Containing Large Amounts of Unidentified Nitrogen

Genus and Species	Family	Nitrogen Unidentified %	NH_3 Nitrogen %	No. of Unknowns Detected
Actinomeris alternifolia	Compositae	36	8.8	0
Althaea rosea	Malvaceae	40	13.6	0
Anchusa capensis	Boraginaceae	38	9.8	0
Aster tanacetifolius	Compositae	36	14.0	1
Bifora americana	Umbelliferae	41	14.4	1
Calendula officinalis	Compositae	37	11.1	0
Corchorus olitorius	Tiliaceae	36	9.8	0
Digitaria longiflora	Gramineae	45	5.4	0
Dimorphotheca sinuata	Compositae	41	10.9	0
Diospyros virginiana	Elenaceae	35	8.1	1
Dithyrea wislizenii	Cruciferae	37	15.5	2
Helichrysum bracteatum	Compositae	40	11.7	0
Heracleum lanatum	Umbelliferae	37	11.4	0
Lunaria annua	Cruciferae	34	10.3	2
Lysicloma desmostachya	Leguminosae	40	11.7	3
Mesembryanthemum crystallinum	Aizoaceae	33	8.9	0
Nasturtiopsis arabica	Cruciferae	33	14.8	0
Ormosia jamaicensis	Leguminosae	46	7.4	0
Osteaspermum ecklonis	Compositae	35	10.5	0
Peganum harmala	Zygophyllaceae	43	7.4	1
Polemonium caeruleum	Polemoniaceae	31	11.0	3
Portulaca oleracea	Portulacaceae	35	12.8	0
Sagittaria papillosa	Alismaceae	32	12.2	2
Selenia grandis	Cruciferae	31	13.0	0
Staphylea pinnata	Staphyleaceae	39	15.9	2

SEED POLYSACCHARIDES

I. Screening for New Seed Sources of Gum

In an effort to find a domestic source of gum to compete with imported gums such as guar, seeds of 110 new species have been surveyed for gum content. Those containing more than 18% water-soluble gum and giving a negative iodine test for starch are shown in Table 1. Two other species should be mentioned which produce seed gums that are alkali soluble, and swell but do not disperse in water. These are seed of Lallemantia royleana, 42873, containing 16.4% gum, and seed of Salvia similata, 41287, containing 10.1% gum.

Eighteen legume seed gums, previously unreported in the literature, were shown to be galactomannans with mannose/galactose ratios between 5 to 1 and 3 to 2. Genera represented were Astragalus, Cassia, Crotalaria, Neptunia, and Sphinctospermum. Specific optical rotations of the gums is inversely proportional to mannose content.

Table 1.--Seeds Containing >18% Gum

Grams 1000 seeds	Species	N-free Gum, % of air-dry seed	CPR	Remarks
144.8	Cassia javanica, 40669	37.2	II	No supply
6.5	Cassia hirsuta, 40667	31.0	II	Toxic*
1.6	Trigonella arabica, 42095	28.3	I	
9.0	Trigonella gladiata, 43067	26.4	II	
4.6	Desmanthus subalatus, 42017	18.9	II	
4.0	Cassia durangensis, 40939	18.3	II	No supply
13.5	Securigera securidaca, 42979	18.2	II	

*When fed at 5% of the diet, rats died.

II. Status of Previously Reported Seeds

Preliminary evaluations of toxicity of a number of gum-bearing seeds have been carried out with the cooperation of WU. The following table comprises species which were selected as promising from the January 1963 report. Further studies await results of plantings.

Table 2.--Status of Seeds Previously Reported

Species	N-free gum, % of air dry seed	CPR	Toxicity* 5% of diet, 56 days	Planted	Remarks
Astragalus hamosus	22.1	Poor	no	'63	
Cassia biflora	25.0	Fair-good	no		
Cassia bonariensis	28.8	Good	yes		
" leptadenia	22.5	Fair-good	no		
" leptocarpa	30.2	Fair-good	yes		

-- continued

Table 2--continued

Species	N-free gum, % of air dry seed	CPR	Toxicity* 5% of diet, 56 days	Planted	Remarks
Cassia occidentalis	26-32	Good	yes		
" covesii	22.3	Fair-good			No supply
" uniflora	28.8	Fair-good	?	'63	
Sphinctospermum constrictum	23.6	Good-excellent		'63	No supply

* Gum is toxic if rats lose weight or die.

III. Analysis of the family Leguminosae

In a study (cooperative with CR) of the overall gum survey the legumes were most closely examined because that family appeared to be the most promising source of seed gum.

Among the Leguminosae as a whole, high amounts of gum (10% or more) are not always accompanied by low amounts (35% or less) of total oil and protein, but in the subfamily Caesalpinioideae high-gum content is associated with low oil and protein (Fig. 1). On the other hand, about 40% of the Papilionoideae samples contain at least 10% gum and more than 35% oil plus protein (Fig. 1). Even though the Papilionoideae does not compare with some of the Caesalpinioideae in gum content, it should be considered because of the much higher proportion of herbaceous species within the subfamily as compared with the largely woody Caesalpinioideae and Mimosoideae. The tribes Trifolieae and Galegeae of Papilionoideae are represented by herbaceous genera. These appear to be promising sources of seed gum. The gum content of such samples ranges from about 7 to 22% (Trifolieae) and from 3 to 27% (Galegeae).

IV. Evaluation of *Cassia marilandica* and *Cassia occidentalis* seed gum

Cassia marilandica gum, separated as described previously by dry milling, was evaluated as a wet-end paper additive. The gum is comparable to commercial guar as illustrated in Fig. 2. Similar results were obtained with both unbleached kraft and bleached sulfite pulps.

Cassia occidentalis endosperm flour (gum) was separated by dry milling. The separated gum comprises 35% of the seed as compared to 27.5% for *C. marilandica* and 23.5% for guar (Table 3). The values for protein and acid insoluble residue in milled gum of *C. occidentalis* are more than twice those of guar gum. This is inherent in the endosperm of the seed and cannot be improved by milling. Nevertheless, the performance of *C. occidentalis* gum as a wet-end paper additive is comparable to guar gum or *C. marilandica* gum (Fig. 2) except for brightness. The color in the *C. occidentalis* gum which lowers brightness is partially caused by adherent bits of dark brown seedcoat. Presumably these could be eliminated by a modification of the milling process.

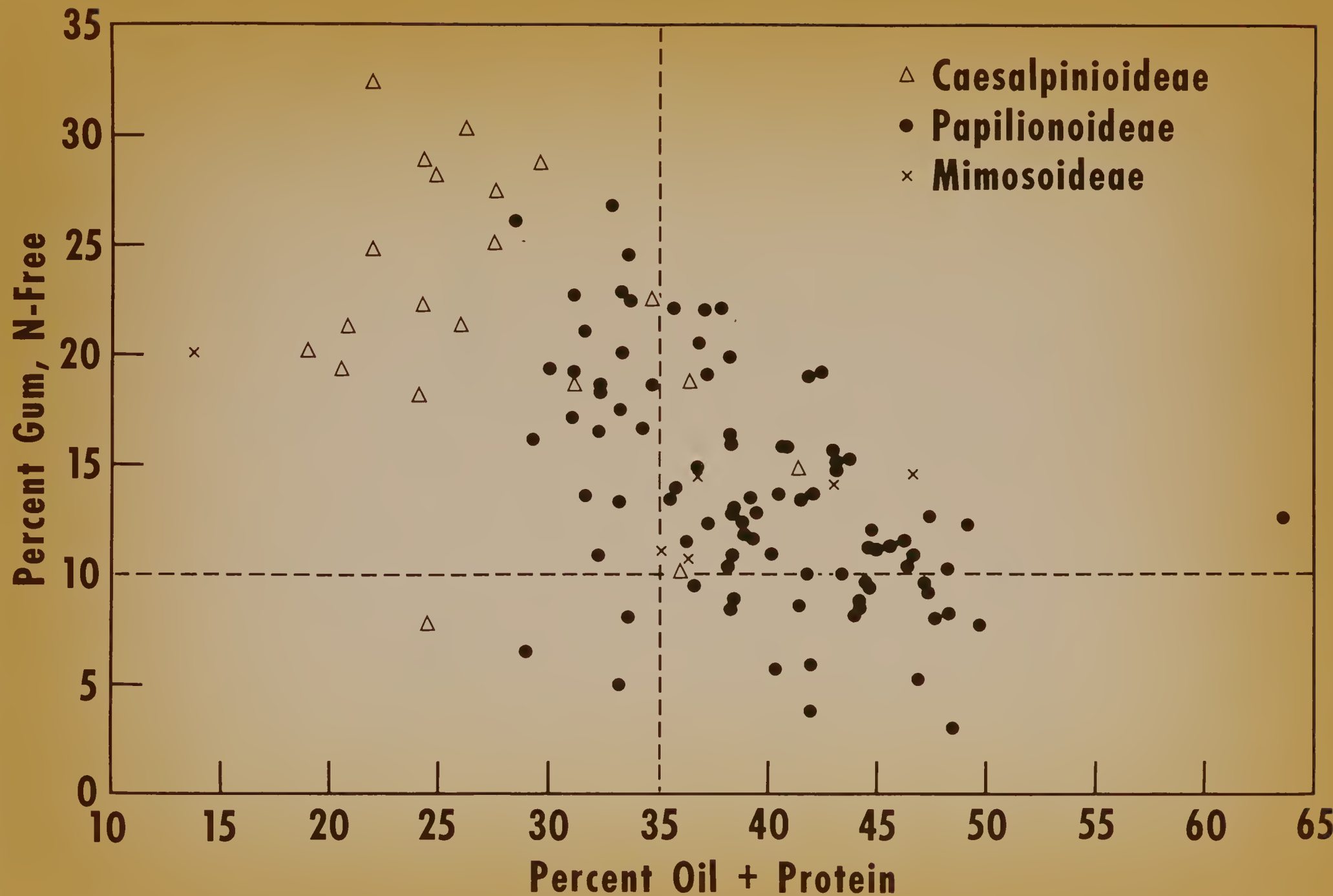

Fig. 1 Relationship of gum content to oil plus protein content in the subfamilies of Leguminosae

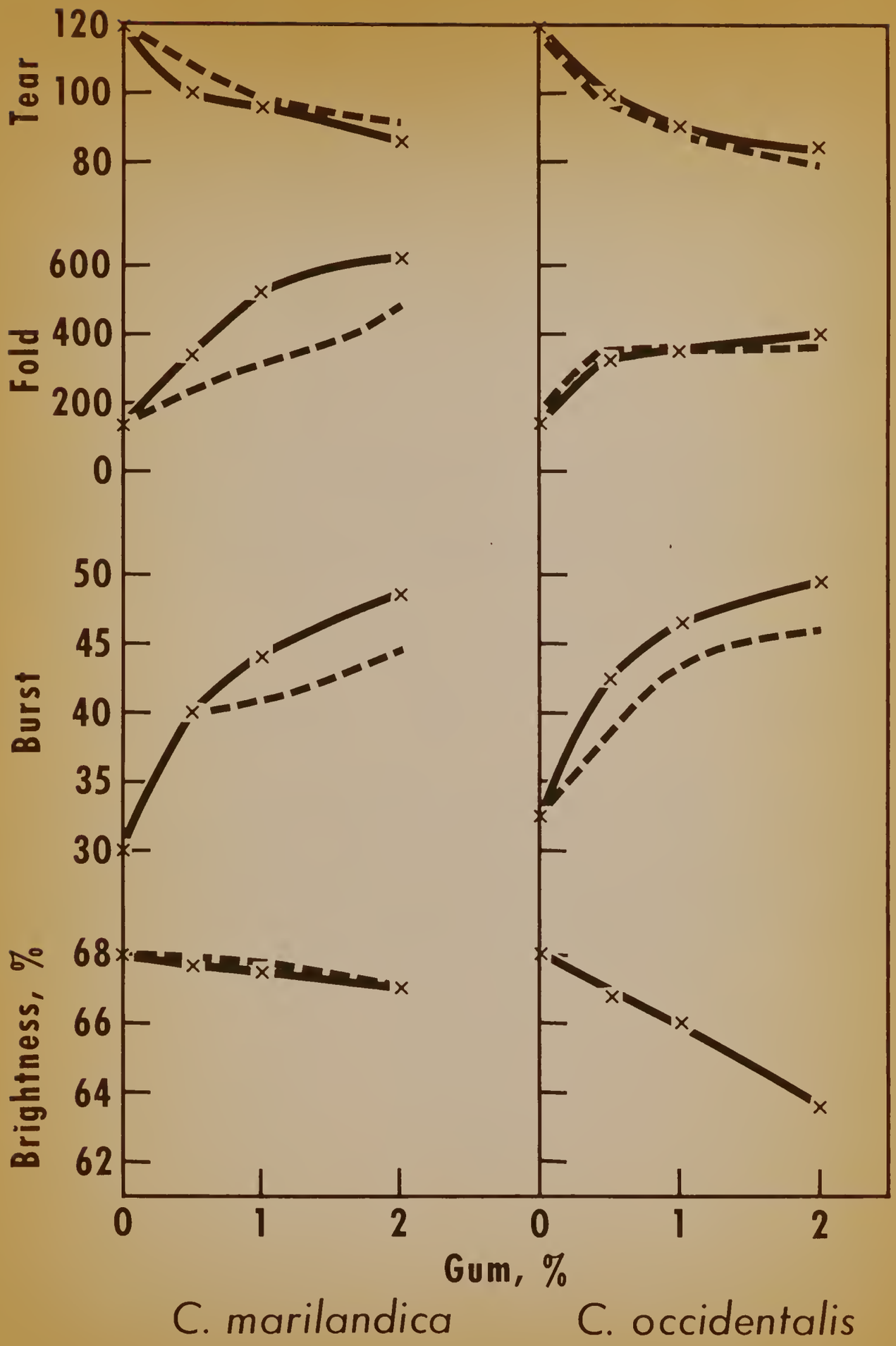

Fig. 2 Effect of seed gums on handsheets of bleached sulfite pulp. No rosin or alum. Guar ----

Table 3.--Analyses of C. occidentalis Seed Fractions

	Percent of seed	Composition of fraction % protein	% acid-insoluble
Seedcoat	23.4	9.3	51.2
Germ	26.2	49.6	21.7
Endosperm	50.4	10.3	12.2
Milled gum	35.1	10.9	12.4

V. Toxicity of Cassia seeds

Some but not all of the Cassias are quite toxic (see Table 2). Cassia marilandica seed is well tolerated by the rat when fed at 5% of the diet (56 days) although some toxic effects were noted at higher levels (Jan. 1963 report). In contrast, C. occidentalis seed killed all rats within 29 days at that dietary level. Autoclaving the seed destroys the toxicity as shown below.

Diet	Rat body wt. at 45 days	Food efficiency
Addis control	159.6	0.207
Addis + 5% C. occidentalis (autoclaved)	159.6	0.206

A recent progress report from Texas A & M (July 24, 1964, PR-2318) indicates that C. occidentalis is toxic to cattle, sheep, goats, and rabbits. The leaves and stems as well as the seed are very toxic. In view of this toxicity, caution in promoting the species as a new crop is in order. Nonetheless, the S-9 committee indicated that the excellent seed yields, agronomic adaptability, and amenability to mechanical harvest make the crop worth further investigation despite seed toxicity to various animals.

FIBER PLANTS FOR PULP

I. Number of Samples Received, Analyzed and Evaluated:

	Total to Nov. 1, 1964	Nov. 1, 1962 to Nov. 1, 1964
Samples received	1216	105
Families	59	0 (new)
Genera	265	10 (new)
Species	534	42 (new)
Samples Analyzed	874 (829*)	135 (90*; 38)
Species	385	34 (29 new)

* Difference between 874 and 829 and between 135 and 90 equals the number (45) of sorghum samples on which the analyses were made on culms and leaf components separately. The 38 is number of new samples exclusive of sorghums (S. bicolor and S. almum) in the systematic study.

Species rated favorably among those examined	100	12 (11 new)

New species with favorable point ratings for pulping potential, based on initial screening:

Species	Family	Source	Rating*
Oxytenanthera abyssinica	Gramineae	Ethiopia	5
Crotalaria eriocarpa	Leguminosae	Mexico	7
Desmodium nicaraguense	Leguminosae	Mexico	7
Eupatorium brevipeticlatum	Compositae	Uruguay	7
Paspalum arechavaletae	Gramineae	Uruguay	7
Dalea leporina	Leguminosae	Mexico	8
Oenothera affinis	Onagraceae	Uruguay	8
Paspalum exaltatum	Gramineae	Uruguay	8
Paspalum haumanii	Gramineae	Uruguay	8
Paspalum quadrifarium	Gramineae	Uruguay	8
Sida rhombifolia	Malvaceae	Mexico	8

* On basis of 5 points (most favorable) to 17 (least favorable).

II. General

Analytical screening has been continued with botanically acceptable accessions obtained primarily from foreign sources. Selectivity in collecting is evident from the predominance of samples in families previously observed as promising. The greater selectivity in collecting is also reflected in a greater proportion of samples and species with favorable ratings.

With reduced activity in primary screening, attention was directed to the potential of selected lines of Sorghum bicolor and Sorghum almum, especially with respect to geographic areas of their adaptability and to the extent of their productivity for pulping applications.

Developmental studies involved continued investigation of kenaf mechanical pulp for resin-bonded hardboard production, mechanical-type pulps for production of selected paper types, and of refining techniques for optimal development of strength properties in kenaf chemical pulps.

Statistical methods have been applied to data related to characteristics of selected fibrous annual plants and properties of their derived pulps to establish possible relationships that might be helpful in assessment of the relative pulping potential of plant species.

III. Fiber screening

Thirty-eight new plant samples representing 34 species, mostly from Mexico and Uruguay, were analyzed and evaluated. Twenty-nine of these species were new to the program. Ten species were legumes; eight, grasses; and four, mallows. Eleven new species based on results of the point-rating scheme have a favorable potential; a twelfth species, a Crotolaria incana from Mexico, had already received a favorable rating on the basis of a domestic sample. Of the new promising species, five are members of the grass family, three are legumes, and one each of the Compositae, Malvaceae and Onagraceae families. Although all analyses have not been completed at this time, it is doubtful that further encouraging results will be obtained from the few remaining samples needing additional characterization. The outstanding species were Oxytenanthera abyssinica, a bamboo from Ethiopia, and Crotalaria eriocarpa, from Mexico. All analytical characteristics of the bamboo were favorable, including a fiber length of 2.0 mm. Cellulose contents of the crotalaria were approaching those of the bamboo, but the combined fiber length was 0.8 mm. in spite of a length of 1.9 mm. for the bast fraction.

Characteristic	Oxytenanthera abyssinica	Crotalaria eriocarpa
Solubles in 1% NaOH, %	29.5	28.1
Crude cellulose, MEA, %	56.7	52.9
Alpha cellulose, %	39.8	36.1
Fiber length, mm.	2.0	0.8 (bast 1.9)
Maceration yield	51.2	49.4 (bast 7.4)

Pentosan and lignin contents have been included in the screening of this reporting period. Both pentosans and lignin contribute to yield, especially in production of mechanical, chemimechanical and semichemical pulps. The amount of lignin present may be used as a rough relative measure of chemical requirements for cooking and bleaching chemical pulps. The Gramineae and Malvaceae were consistently lower in lignin content and the Gramineae higher in pentosan content than the other families.

IV. Sorghums

Following the preliminary evaluations of 128 sorghum samples discussed in the previous report, 9 lines were recommended to CR for further field study to ascertain their relative merits when grown in widely-scattered geographic areas. These lines comprising a forage type, two grains, a broom, a durra, two almums and two kafirs were grown at six locations in 1963. Stations involved were located at Chillicothe and College Station, Texas; Experiment, Georgia; Lafayette, Indiana; Ames, Iowa; and Rosemount, Minnesota. Appraisals of pulping potential were based on proportions of stalks not including heads, leaf-culm relationships, and composition of both leaf and culm fractions with respect to ash content, alkali-insoluble matter, crude cellulose and alpha cellulose. These data integrated with crop yield values furnished by the agricultural experiment stations (through CR) led to more reliable bases for selection of sorghums as potentially useful pulping materials.

Ash contents in leaves including the leaf sheath were significantly greater then in the culms of corresponding samples. This could detract from the utility of the leaf portion for quality pulps. Excluding samples for Chillicothe, Texas and Lafayette, Indiana, the content in the leaves ranged from 3.1 to 13.1% while the values for the culms were 1.6 to 6.1%. On an overall average, little difference exists between leaves and culms in the alkali solubility, crude cellulose and alpha cellulose. However, an individual samples approximate differences ranged from 7 to 12% for alkali solubility, 6 to 9% for crude cellulose and 8% for alpha cellulose. Where such differences occurred they generally favored the culm fraction.

Dimensional characteristics and macerate yield values for the leaf and culm fractions of sorghums provide some indication of the relative merits of these two components for pulping. Data for these factors in the components of the sorghum grown in Iowa in 1963 are tabulated below:

Material	Fiber length, mm.		Fiber width, average microns	Lumen width, microns	Cell wall thickness microns	Maceration yield, %
	Av.	Max.				
Sorghum almum PI 190579						
Culms	1.04	7.2	15.4	5.3	5.1	56.5
Leaves	0.57	3.5	10.5	5.0	2.7	44.9
Sorghum bicolor PI 229837 (kafir)						
Culms	1.20	5.9	16.7	7.4	4.7	51.4
Leaves	0.64	4.3	10.4	4.9	2.8	37.2

The greater fiber length and wall thickness of fibers from the culms contribute to better strength characteristics in pulps than the short, thinner-walled fibers from the leaves. While many leaf fibers are about 3 mm. and longer the predominance of small (0.1 mm.) cellular material in the leaf fractions detract

from the effective or mean fiber length. Widths of both leaf and culm fibers are in the narrow category that would be useful in producing smooth papers. While the macerates of the leaf components contain fibers of appreciable length, the preponderance of small cellular matter would give rise to very tight papers while causing a reduction in pulp freeness. This last characteristic caused by the leaf fraction would necessitate slower machine speeds and greater screening areas. On the basis of these observations sorghum lines with limited foliage would be preferred for pulping. For the two samples considered here the macerate yields from the culms are appreciably greater than those from the leaves.

The combined field and laboratory data show that productivity of useful components in the 1963 crop is greater in those sorghums grown in the Midwest than in the South or Southeast. Samples from Minnesota had abnormally high moisture contents that indicate too short a season or need for artificial drying before storage.

Lines with favorable characteristics include the two kafirs (PI 229837 and 229847), the broom (PI 177549) and the two almums (PI 190579 and 202410). The durra (PI 179749) appeared sufficiently promising in the Iowa planting that this line should also be considered for further study, at least in Iowa. Gross yields of solids, moisture-free basis, in the Iowa plantings ranged from 8.2 to 12.2 tons per acre while cellulosic matter theoretically recoverable (basis analyses) in a pulping operation ranged from 3.65 to 5.33 tons per acre. On the basis of prefrost samplings, the Indiana plantings were less productive; however, estimates based on postfrost yield measurements indicate that productivity at the Indiana and Iowa stations might be comparable. These yield values meet the tonnage requirements estimated earlier by Trotter. Estimates of cellulosic material may be enhanced in pulping operations by hemicellulose contents not determined for these sorghums.

V. Statistical evaluations

In an earlier phase of the fiber screening program 11 selected species from the monocotyledonous and dicotyledonous groups had been subjected to small-scale pulping by the sulfate process. Species involved in this study comprised the following:

Monocots	Dicots
Andropogon virginicus	Cannabis sativa
Lygeum spartum	Hibiscus cannabinses
Miscanthus sinensis	Crotalaria juncea
Pennisetum glaucum	Hibiscus esculentus
Sorghum almum	Sesbania sonorae
Stipa tenacissima	

Both raw materials and derived pulps were characterized chemically and physically as extensively as was practical at the time. The measurements of 76 characteristics of raw materials and derived pulps provided the basis for a statistical study by Biometrical Services to determine the degree of correlation between properties of raw materials and products. The data were obtained from 21 sets of observations, that is, 21 pulping conditions. Seventeen of the measurements concerned properties of the plant species, and 59 measurements described the derived pulps at various stages of processing. One thousand three correlations were computed between independent (raw materials) variables and the dependent (products) variables; 1711 correlations between dependent variables; and 136 correlations between independent variables.

Total correlations concern relationships between characteristics of the raw materials and of the products without regard for possible differences that might be attributable to class, that is, monocot or dicot. If the difference between the class is large, it is possible to have a statistically significant total correlation yet have no within-class correlation. In this study the data associated with the two classes tend to fall into two fairly distinct groupings. In many instances there was no evidence for within-class correlation between variables.

This study has shown that many of the dimensional characteristics of the plant fibers might be used interchangeably without altering conclusions appreciably. A high degree of correlation was also observed for a number of dependent variables--strength characteristics of the pulps--however, the expected frequency of correlation throughout the range of the refining treatment was not evident for the pulps.

This statistical analysis has indicated the following independent variables studied for the raw materials as being most significantly correlated with the measured characteristics of the product and offer a possible means for predicting the quality of plant species with respect to their pulping potential:

Crude cellulose (MEA) content
Alpha cellulose content
Pentosan content
Lignin content
Length, bast and woody fibers
Width, bast and woody fibers
Lumen width or cell wall thickness
Fiber maceration yield

These are factors that have generally been measured in the fiber screening program, and most have been used in the point rating scheme. These data have been supplemented by other data according to the plant species under consideration. Thus, in the systematic evaluation of sorghums, pith content and the leaf-culm ratio are important in the appraisal.

VI. Developmental

Kenaf pulps for papers

Mechanical and closely associated chemimechanical pulping techniques have been used advantageously in processing kenaf to high-yield, high-opacity pulps, especially for publication-grade papers. These techniques avoid the compromise involved in chemically pulping the combined components of a bimodal fiber system to a near optimal condition. A sequence of steaming, classifying and refining treatments developed at NU produces kenaf pulps that have good opacity as well as good bulk and strength properties. Semibleached pulps prepared from clean chopped kenaf by steam and mild neutral-sulfite treatments were produced in yields of about 70-73%. This represents an improvement in yield of about 70% over those that have been obtained for kenaf bleached sulfate pulps of 75 to 80% brightness. The pulp from a 2% neutral-sulfite treatment was slightly stronger than from a 4% neutral-sulfite treatment or from steaming. All three pulps were used in preparation of handsheets from blends with kenaf bleached sulfate pulp. Various formulations based on 100% kenaf fibers were used to produce newsprint, and bond-type papers with properties, brightness excepted, generally superior to their commercial counterparts. The book paper also had good quality characteristics. Sufficient margin for opacity and strength exists that increase in bright to desired values might be anticipated while still meeting the opacity and strength requirements. Experiments in bleaching after refining and in bleaching with a bisulfite sequence indicate that attainment of the necessary brightness levels appears promising without brightness losses experienced after the more customary bleaching treatments. Topochemical treatments on refined pulps are expected to reduce the present high (25 to 30%) bleach requirements needed to exceed 60 brightness with these mechanical and chemimechanical pulps. A bleaching sequence used with a 4% neutral-sulfite chemimechanical pulp to attain 60-plus brightness comprised the following stages: (1) chlorination with 5% chlorine; (2) 2% sodium bisulfite treatment; (3) hypochlorite treatment (5% Cl_2 equivalent) at pH 6.0-6.5; (4) hypochlorite treatment (5% Cl_2 equivalent) at pH 9.0; and (5) chlorine dioxide (6% Cl_2 equivalent) at pH 4.5. At the end of this sequence the freeness of the pulp had dropped from 415 ml. to 350 ml. Schopper-Riegler. A yellowish tint persists in the bleached mechanical and chemimechanical pulps. Ability to develop high-yield, high-opacity pulps from kenaf could contribute to production of papers of lower basis weight and with smoother surfaces. The first would result from absence of inorganic fillers frequently used in papers to develop acceptable opacity. The second, resulting from fine narrow fibers would reduce the quantity of surface coatings needed to develop the smooth finishes desired for improved printing and appearance.

Difference in row spacings (7 and 28 inches) for Nebraska-grown kenaf had no apparent effects on the pulping potential of the two kenafs as judged by pulp yields and pulp properties. Average stalk diameters at mid height were greater (1/2 inch vs. 5/16 inch) for the stalks grown with the greater row-spacing. Sulfate pulps prepared from these kenafs were essentially the same in properties and comparable to kenaf pulps in general prepared under similar conditions.

Kenaf for structural boards

Steam-cooked, mechanically-defiberized pulp from kenaf was produced in 88% yields by the NU technique for preparing resin-bonded hardboards. The technique involves cutting stalks to closely-controlled lengths (1/2 inch); removing the dark parchmentlike bark in a milling-screening sequence; steaming to soften bast fibers and woody material; defiberizing in a disk refiner; classifying with an impact-type wet screen; and refining the fractions to appropriate characteristics. By these treatments, clean pulps free of shives with controlled bulk and drainage characteristics can be prepared. Recovery of pulps after refining approximates 78%. For preparing wet-formed mats from the pulps 19% excess phenolic resin is used to obtain retentions of 10 and 20% contents in final boards, smooth on both surfaces. Control of moisture content and resin advancement prior to hot pressing was obtained in part by preconditioning wet mats with combinations of vacuum, oven-drying and dielectric heating. Tensile values from individual resin-bonded boards at 10 and 20% resin levels have reached 11,000 p.s.i., although minimum values within the same board were of the order of 6,000 p.s.i. Experimental resin-bonded wood pulp boards produced by one commercial firm have been reported to have tensile strengths to 12,000 p.s.i. Moisture and other volatiles in 10% resin boards of approximately 10,000 p.s.i. or greater tensile ranged from 4.1 to 7.3%; in 20% resin boards from 2.0 to 3.4%. Many boards, although of satisfactory appearance, possessed much lower tensile values. The effect of moisture and volatiles, however, was not consistent, blistering of boards sometimes occurring at lower moisture levels. To reduce the possibilities for localized concentrations of moisture in wet mats as formed, a pulp spreader was used to distribute pulp as uniformly as possible over the surface of the mold screen. Despite this precaution differences in dry solids contents within quarter-sections of the mat ranged up to almost 7%. Such differences would account for difficulty in control of moisture contents within the ranges noted above.

Refining techniques for kenaf chemical pulps

Experience in processing fibrous annual plants to chemical pulps for papermaking have disclosed that development of strength properties is very rapid, but the range in improvement is rather limited and is accompanied by hydration, increasing the "wetness" of the fibers. Rapid and easy development of pulp properties is economical from the standpoint of power expended and in-process space requirements. A wet pulp, however, is slow draining and will need greater screen areas in thickeners, classifier screens and paper machine wet ends.

In a comparison of some refining techniques involving a conventional beater and ballmills made from stainless steel and a ceramic porcelainlike material, it was observed that fibrils developed on the exterior of individual fiber during the early stages of refining were broken off as the refining progressed. This formation and fragmentation was intermediate to that of the hard and softwood pulps, this behavior being very pronounced in the hardwood pulp as measured by the increase in "fines." This behavior in the kenaf pulp would also account for the rapid increase in "fines" as refining progressed.

Equivalent drops in pulp freeness were obtained after 10 minutes in the beater, 40 minutes in the ceramic ballmill with porcelain balls, and 120 minutes in the stainless-steel ballmill also with porcelain balls. Although the tear factor decreased more rapidly with the metal ballmill than with the ceramic mill, the burst factor increased more rapidly. The same general magnitude for breaking length and folding endurance was realized with both mills, but results with the metal mill were erratic.

For kenaf bleached sulfate and softwood bleached kraft the bursting strengths were of the same general magnitude whether from the beater or ballmill, although the results from the ballmill treatment were consistently but slightly less. Tearing resistance of the kenaf pulp, although significantly higher than that of the hardwood pulp, does not show significant difference attributable to differences in these two refining techniques. The hardwood is influenced more favorably by ballmilling than by beating. Beater refining of the softwood pulp contributes significantly to greater tear resistance than does ballmilling. This effect might result from action of the balls on cell wall structure, whereas beater refining tends to cause more reduction in fiber length. Good resistance to tear is associated with thick cell walls. The softwood pulp and to a lesser extent the hardwood pulp show marked improvements in resistance to tear in the initial stages of refining followed by a rapid drop as refining progresses. Kenaf pulp has not yet demonstrated this effect in the early stages of refining.

With respect to breaking length (tensile strength) beater-refined kenaf pulp exhibits a slower buildup than ballmilled pulp. The loss in strength on continued refining is also later with the beater than with the ballmill. Strength development for hardwood pulp is more rapid with ballmill than with beater although the ultimate development is of the same magnitude. Development of tensile strength in softwood pulp requires more refining by both beater and ballmill than in kenaf pulp. The development in the softwood pulp was greater by the beater than by the ballmill.

Observations for fiber length of pulps at various stages of refining, although being somewhat erratic, indicated that fiber length tended to remain greater for ballmill-refined pulps than for beater-refined pulps.

Results of this refining study suggest that a refining technique for kenaf chemical pulps should involve limited or controlled exposure to fibrillating action to avoid removal and fragmentation of fibrils. Pump-through refining techniques might meet this need better than beater refining which is performed batchwise. Where significant differences in thickness of bast and woody fibers exist, processing of the separate fiber types may be necessary to achieve the necessary control.

Industrial interests

Kimberly-Clark Corporation has planted kenaf for two seasons now for experimental studies in the pulp mill at its Coosa Pines, Alabama operations. Approximately 13 acres (this was acreage harvested) were planted in 1963 but plantings were

not well timed to provide the anticipated per acre yields. In 1964, with more time for arranging seed procurement and soil preparation, 27 acres have been planted to kenaf. A half-acre has also been planted to crotalaria (sunn hemp).

According to a letter of Dr. G. B. Killinger (10/16/64) of the Florida Agricultural Experiment Station at Gainesville, the Hudson Pulp and Paper Company, Palatka, Florida, has requested of the Florida Station sufficient seed of Everglades 41 kenaf to plant from 10 to 100 acres in 1965. Acreage of this magnitude is contemplated to provide enough kenaf to operate the pulpmill for 1 or 2 days. Information is also needed regarding the period over which the crop could be harvested for pulping purposes.

This paper company has also contacted NU directly for information regarding kenaf pulping.

REPORT OF CROP DEVELOPMENT RESEARCH
Chemurgic Crop Investigations
New Crops Research Branch
Crops Research Division

Introduction

Vernonia

In the 1963 season, experimental plantings were made in approximately 35 locations in 24 states. Seed yields at Glenn Dale, Md., were 2,000 pounds or more per acre on the better plots. Several plantings were made to increase seed for pilot-plant commercial extractions. However, these were generally unsuccessful. Attention is being given to plant selection for crop improvement breeding programs. The undesirable characteristics of seed ripening over an extended period, plus seed shattering at maturity will have to be modified. Another approach to this problem may be the development of methods for repeated harvesting at intervals during the season.

Crambe

During 1963, more than 40 tons of Crambe seed were produced by growers in six Western states for the pilot-plant extraction and chemical research program of NURDD. Seed yields up to 2800 lbs/A were obtained. In 1964 the number of growers was reduced to simplify production problems. One grower in Oregon had an exceptionally good crop with a total production of 27 tons from 25 acres. His production was spread among 4 different fields differing in productivity and it was estimated that the best fields yielded over 3,000 pounds per acre. The two growers in eastern Wyoming had total failures because of the severe drought in that area. One grower in eastern Washington had a moderate crop under dry land conditions. One grower in Wisconsin had a fair crop for his first experience although the final yield will not be known until his seed is received. Approximately 50 tons of seed were produced in 1964. Plantings of several thousand acres are anticipated by private concerns in 1965.

Limnanthes

Seed of 24 Limnanthes accessions were planted at Glenn Dale, Md., on October 19, 1962. Seed yields varied from 42.9 to 615.6 pounds per acre. The top yielders were:

PI 283705	*L. alba* var. *versicolor*	529.7	lbs/A
PI 283712	*L. douglasii* var. *nivea*	486.8	lbs/A
PI 283713	*L. douglasii* var. *nivea*	615.6	lbs/A
PI 283724	*L. gracilis* var. *parishii*	572.7	lbs/A

These results suggest further tests to evaluate row widths and within-row densities.

Results from California were not as encouraging as seed yields ranged from 0 to 50 pounds per acre.

Limnanthes douglasii (PI 278170) produced the equivalent of 2,235 pounds of seed per acre in Alaska. The comment was made that heavy losses occurred as a result of seed shattering. The small stature of the plant may also complicate mechanical harvesting.

Petroselinic acid sources

The 1963 spring plantings of fennel were for the most part unsuccessful. An observational planting at Ames, Iowa, produced no seed in 1963 but overwintered. Yields obtained from Indiana were as follows:

Row width	N level	Seed yield/A
14"	0	669
14"	50	506
38"	0	535
38"	50	392

Stands in fall or early spring plantings (1964) in Texas and Oklahoma were satisfactory but seed yields will be low. Results from experiments in Oregon and California are not available.

Fennel seedstocks now available for planting purposes do not appear to have much potential in NC-7 states however judicious use of insecticides and perhaps herbicides might improve yields. Of considerable concern is the inconsistency of this plant. If fennel is to be handled as an annual, earlier maturing lines are needed for north central areas. It is suggested, provided the industrial potential for petroselinic acid is sufficient to justify further agronomic effort, that related species and perhaps a few new fennel seed sources be tested.

Annual pulp crops

Yields from 1964 tests are not available for inclusion in this report. With some exceptions, prospects are better than for 1963. Plantings of special interest include:

a) Kenaf and sunn hemp (2 1/2-3 acres of each) at Experiment, Georgia. Material is to be consumed in a harvesting and storage test in cooperation with the Northern Utilization Lab.

b) Kenaf (1 acre) at Gainesville, Florida. Paper company representatives have been invited to observe the planting and will be offered material from it. The yield should be excellent.

c) Kenaf (27 1/2 acres) and sunn hemp (1/2 acre) plantings by Kimberly-Clark. Morning glories pose a rather serious problem.

d) Kenaf in North Carolina - row-width x plant density study.

e) Kenaf and sunn hemp in Nebraska - herbicide test.

f) Sorghums - activity greatly curtailed because of low seed supply. Comprehensive tests are being conducted at the Iowa and Illinois stations.

Preliminary Evaluations

See following tables for details.

Preliminary Evaluation - Southern Region (S-9), 1963.

PI NO.	Species	Arkansas	Georgia	Florida	Oklahoma	Texas
279691	*Cassia alata*	L	L	D	L	O
279692	*C. leptadenia*	DL	DS	D	PS	D
279693	*C. leptocarpa*	LD	DL	D	L	L
279694	*C. occidentalis*		LS	LS	PS	LS
279695	*Crotalaria longirostrata*	PS	L	O	S	S
279696	*Crotalaria* sp.	L	L	O	L	L
279700	*Schkuhria wrightii*	PS	O	O	O	O
279715	*Ipomoea* sp.	S	LS	D	DS	PS
279722	*Salvia texana*	O	PD	D	PS	O
288247	*Cassia uniflora*		L			
288248	*Cuphea painteri*		O			
288249	*Dalea nutans*		L			
288250	*Physalis nicandroides*		O			
288258	*Cassia marilandica*		L			
288259	*Doronicum caucasicum*		O			
279698	*Ipomoea parasitica*	S			S	
279699	*Polanisia viscosa*				O	
280049	*Solanum aviculare*				PS	
279702	*Zaluzania discoidea*				P	

Legend:

- O No germination
- P Poor stand
- D Poor development
- L Late or no flowering
- S Small amount of seed collected
- G Good crop potential

Preliminary Evaluation - Ames, Iowa and NC-7, 1963.

PI NO.	Species	Results and comments
279692	Cassia leptadenia	A low growing bushy plant. Bloomed too late to produce mature seed.
279693	C. leptocarpa	A large bushy plant that was very vigorous. Bloomed late and no pods were formed.
279694	C. occidentalis	Only a few pods formed before frost. No mature seed.
279696	Crotalaria sp.	Leaves yellowed. Started blooming about August 1 but blossoms aborted and no pods were formed until after October 1.
279705	Cichorium intybus	Seed obtained; probably require a low temperature induction because of rosetting habit of many plants.
279717	Lunaria annua	No seedlings obtained.
279710	Commandra pallida	No stand.
	Lallemantia iberica	No stand
	L. peltata	Good vigor and uniformity, but serious shattering problem.
279706	Sideritis montana	Good stand, however it does not appear very productive.

- -

279690 Calendula officinalis

Since flowering is indeterminate, multiple harvests will probably be necessary to obtain reasonably good seed yields. Hand-harvested plots in Minnesota produced 277 pounds per acre. This species does not appear promising.

271451 Raphanus raphanistrum

This plant produced pods that may be up to 21 inches long. Seed yields of 848,782, and 868 pounds per acre were obtained in Kansas, Indiana, and Minnesota, respectively. Plants are vigorous and stands are usually good, however some lodging has been noted.

Preliminary Evaluation - Pullman, Washington, 1963.

PI NO.	Species	Characteristics*	Comments
226649	Satureja hortensis	SG	Indeterminate flowering but good seed retention. Plant erect. Yield of 2500 lbs/A.
279700	Schkuhria wrightii	O	
279708	Amblyolepis setigera	O	
279717	Lunaria annua	L	Fair stand. No flowers October 31.
279720	Mercurialis annua	PS	Plants decumbent to erect. Pods drop as mature.
288246	Cassia covesii	PS	Seed of poor quality.
288247	C. uniflora	PL	Plants 8 to 10 inches tall. Few flowers appeared in early October.
288248	Cuphea painteri	O	
288249	Dalea nutans	PD	Stand decreased with time. No flowers.
288250	Physalis nicandroides	PDS	Plants were chlorotic and deformed. Seed obtained from seemingly normal plant.
288251	Helichrysum fulgidum	PL	No flowers by October 31.
288252	Peucedanum capense	O	
288253	Astragalus hamosus	L	Plants prostrate and dense.
288254	Crepis foetida	L	Seed shatters easily. Good stand.
288255	Onobrychis argyrea	PL	No buds on October 31.
288256	Thlaspi perfoliatum	D	Good early stand but plants died when about 1" in height.
288257	Oenothera drummondii	LS	Excellent stand, plants to 36" tall. Lodged.
288260	Echium plantagineum	S	Excellent stand, indeterminate flowering with seed shatter. Has irritating hairs and volunteers.
288261	Iberis amara	SG	Excellent stand; plants 8-10" tall and erect. Good seed retention. Mechanical harvest possible.

*Legend:
- O No germination
- P Poor stand
- D Poor development
- L Late or no flowering
- S Small amount of seed collected
- G Good crop potential

Preliminary Evaluation - Chico, California. 1963.

PI NO.		Characteristics*	Comments
288246	Cassia covesii	PDL	A single plant did not flower.
288247	C. uniflora	PS	Flowers indeterminately.
288249	Dalea nutans	PD	Seedlings did not survive.
288250	Physalis nicandroides	O	Special treatments may be required for germination.
288251	Helichrysum fulgidum	PL	Good germination in Petri plates but not in soil.
288252	Peucedanum capense	O	Very poor seed.
288253	Astragalus hamosus	PD	Plants are low rosettes and no flowers appeared.
288254	Crepis foetida	P	Plant survival poor and plants very small, frost tolerant.
288255	Onobrychis argyrea	O	
288256	Thlaspi perfoliatum	L	Good germination, plants to 36" tall, lodged badly, sensitive to frost.
288257	Oenothera drummondii	PDL	Prostrate habit, no seeds produced.
288259	Doronicum caucasicum	O	
288260	Echium plantagineum	S	A very agressive plant that could become a weed, indeterminate flowering; leaves, bracts, and stems covered with spiny epidermal hairs which makes the plant very unpleasant to work with.
288261	Iberis amara	S	Indeterminate flowering but seeds persist. Plants small.

*Legend:

- O No germination
- P Poor stand
- D Poor development
- L Late or no flowering
- S Small amount of seed collected
- G Good crop potential

Preliminary Evaluation - Geneva, New York. 1963.

PI NO.	Species	Results and comments
279724	Thalictrum dipterocarpum	No germination.
279726	Valeriana officinalis	Uniform, semi-erect, vigorous plants. Ripe seed harvested on July 26. Seeds bear tufts of hair.
279727	Veronica spicata	Uniform, erect, and vigorous. Bloomed July 5. Very small seed.
288256	Thlaspi perfoliatum	Prostrate with poor vigor. Half of the plants were dead by August 6, and the others failed to flower.
288261	Iberis amara	Uniform, erect, and exceptionally vigorous. Bloomed June 15. Seed harvested August 6. Known as white candy tuft. Seed capsules resemble those of shepherd's purse.
288262	Lobularia maritima	Medium erectness. Vigorous. Flowered June 20, and harvested on August 10.

CRAMBE SUMMARY - 1963 EXPERIMENTS

Location	Size - Planting P = Small Plots	Soil Type	Last Spring Frost	Total Rain + Irrig.	Fertilization	Seeding Date	Seeding Rate	Row Spacing	Depth of Planting	Seeding Equipment	Pest Damage	Harvest Date	Harvest Method	Av. Yield in Hull	Av. Height at Harvest
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	acres			in.			lb/A	in.	in.					lb/A	in.
Pennsylvania Landisville	P	silt loam	5-24		240# 8-16-16	4-17	6.6	12	0.5	hand	0	8-5	hand	377	
Minnesota Rosemount	P	silt loam	5-23	12.5	fall 300# 0-12-36	4-13	10.0	6	1.0	Planet, Jr.	0	8-2	hand	803	41
Iowa Ames	P	silty clay loam	5-22	25.1	variable	4-6	52.0	12	1.0	Funnel Seeder	0	7-19	hand	1297	33
Indiana Lafayette	P	silty clay loam	5-22	12.8	150# 18-46-0	4-15	15.0	14	1.0	Planet, Jr.	0	7-20	hand	1782	35
Oregon Pendleton	P	silt loam	3-6	5.3	40#N fall	3-6	10.0	12	1.0	Belt Type Nursery Planter	0	8-19	Cut - Dried Nursery Thresher	1051	36
Albany	8.6	clay	3-17		5-16 240# 16-24-0	5-17	12.0	12	1.5	Oliver	Symphlids	9-25	Cockshut Combine	2102	48
Corvallis	P	clay loam	4-2	7.5	6-6 300# 16-20-0	5-20	10.0	12	1.0	Planet, Jr.	Pheasant on Seedlings	8-13	Plot Thresher	2284	45

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	acres			in.			lb/A	in.	in.					lb/A	in.
Colorado Kersey	5	heavy clay	5-20	6.0	4-20 200# 18-46-0	4-26		22	1.5	J.D. No.66 B&B	Lygus Bugs	9-15	J.D. No.95 Self-Pro. Combine	452	36
Ft. Collins	8	loam	5-20	17.6	0	4-16		20	1.5	J.D. Sugar-beet Planter	Pigweed & Foxtail	9-15	Swathed & combined	124	36
Wyoming Pine Bluffs (Prosser)	7	loam	5-21		0	4-26		27	1.0	J.D. Flexi-Planter	Few insects Many weeds	10-17	J.D. 12A combine	240	18
Pine Bluffs (Moritz)	6	sandy clay	5-21		0	4-25	5.0	20	2.0 too deep	J.D. Flexi-Planter	weeds	9-2	Combine	1416	24
Albin	10	loam	4-27	2.3	0	5-2		10	1.5	J.D. Drill	weeds	8-12 8-15	Swathed & combined	500	24
Egbert	5	sandy silt loam	4-28	9.0	0	5-1		10	0.75	McC D. 10"x14"	weeds	8-31	Swathed & combined J.D.95	2328	30

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	acres			in.			lb/A	in.	in.					lb/A	in.
Wyoming Torrington	0.8	sandy loam	5-13		0	5-3		22	0.75	Sugar-beet Planter	weeds	10-21	A.C. 90	1750	30
Cheyenne	P	clay	5-22	?	0	5-17	8 seed per ft.	18	?	Planet, Jr.	Few Lygus Bugs Flea Beetles	10-17	Hand cut Plot Thresher	1833	34
North Dakota Park River	2	L.C.	5-21		90# 24-20-0	5-16		30	1.5		Flea Beetles	8-9 9-5	Swathed Case A-6 Combine	650	33
South Dakota Watertown	P	Kranz-burg	5-23	ca.: 15.0	last fall 60-40-0	6-5	9.0	18	0.5	Home-made	weeds	8-21	IHC Combine by hand	2070	23
Brookings	P	Vienna	5-22	ca.: 20.0	0	5-8	21.0	18	0.5	Home-made	weeds	8-12	IHC Combine by hand	1259	20
Washington Pullman	24	Palouse silt loam	5-4	4.09	0	5-18	12.0	7	1.5	John Deere	Penny Cress & Wild Oats	9-6	IHC 151 Combine	500 to 600	36

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UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
Crops Research Division
New Crops Research Branch

VERNONIA RESEARCH SUMMARY - 1963[1/]

Compiled by
George A. White and J. R. Haun
Chemurgic Crop Investigations

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[1/] This report summarizes 1963 cooperative crops research on Vernonia as a source of epoxy fatty acids. Publication, display, or distribution of any data or statement herein should not be made without prior approval of the New Crops Research Branch, CR, ARS, USDA and the cooperating agency or agencies involved.

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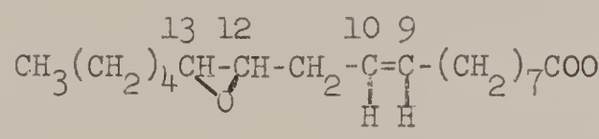
INTRODUCTION

The purpose of this report is to summarize the agronomic and analytical results that were obtained from 1963 experimental plantings of Vernonia anthelmintica (L.) Willd. By presenting the current crop development status, future research efforts can be directed toward specific problems which hamper the successful establishment of Vernonia as a new crop.

A brief consideration of the epoxy or related components of Vernonia oil should answer some of the questions that are posed by interested researchers. About 70 per cent of the oil is composed of glycerides of vernolic (epoxyoleic) acid -



Trivernolin, a triglyceride, is the most prevalent and most desirable of these glycerides; but, upon activation of the lipase enzymatic system, it is degraded to vernolic acid and 1, 3 divernolin. Grinding or flaking in preparation for oil extraction activates the lipase system, however suitable methods are available to prevent this activation.

Apparently, the content of vernolic acid is related to seed maturity. Immature seeds are usually low in oil and epoxy oxygen content but high in vernolic acid. In addition, the same characteristics generally apply to seed that has been subjected to unfavorable conditions during late stages of maturity.

Naturally occurring epoxy fatty acids have been found in other plants and may be more widespread than previously thought. For example, vernolic acid has been found in the seed oils of kenaf (Hibiscus cannabinus L.) and okra (H. esculentus L.).

In results from seed analyses, the percentages of vernolic acid and trivernolin have been reported as free fatty acids and saponifiables (or esters) respectively. Henceforth, these components will be discussed as free fatty acids (FFA) and saponifiables.

To concurrently interpret yield and analytical results, the following model, which represents a desirable range of various constituents of Vernonia seed is given:

Oil content (%) - - - - - - - - - - 26.0 to 30.0 (moisture free basis)
Epoxy oxygen in oil (%) - - - - - 3.80 to 4.00
Free fatty acids in oil (%) - - - 1.00 to 2.00

Pakistanian seed (plant introduction number 283729) was used in experimental plantings unless otherwise noted.

1963 FIELD RESULTS

California - Davis

Vernonia (PI 263368) was planted in an unreplicated test at approximately monthly intervals from the period of December 5, 1962, to October 4, 1963. Plants were carefully cut by hand and threshed.

Date planted	Emergence	Flowering First	Flowering Peak	Harvest date	Seed Moisture	Seed Oil	Oil constituents Epoxy oxygen	Oil constituents FFA
					%	%	%	%
12-5-62	none							
1-8-63	none							
3-2-63	poor	6-20	7-18	9-15	4.73	25.5	3.68	2.65
4-1-63	poor	6-20	7-18	9-15	4.61	24.2	3.60	2.28
5-1-63	good	7-1	8-19	10-4	4.95	24.6	3.74	2.39
6-4-63	good	8-25	9-10	11-15	4.86	20.9	3.86	1.90
6-29-63	good	9-10	10-10	11-15	4.76	21.0	3.82	2.95
8-4-63	good	10-5	frost					
9-10-63	good	frost						
10-4-63	none							

Oil content decreased with delayed planting while epoxy oxygen increased slightly but free fatty acids varied.

Georgia - Experiment

Harvesting of plots was interrupted by rain and high winds, thus considerable seed was lost by shattering and yields were not determined. Sample weights from some plots that were harvested before adverse weather indicated yields of about 900 pounds per acre. An unidentified wilt killed a few plants.

Seed of excellent quality was obtained from this planting. None of the analytical factors were affected by row width or plant density.

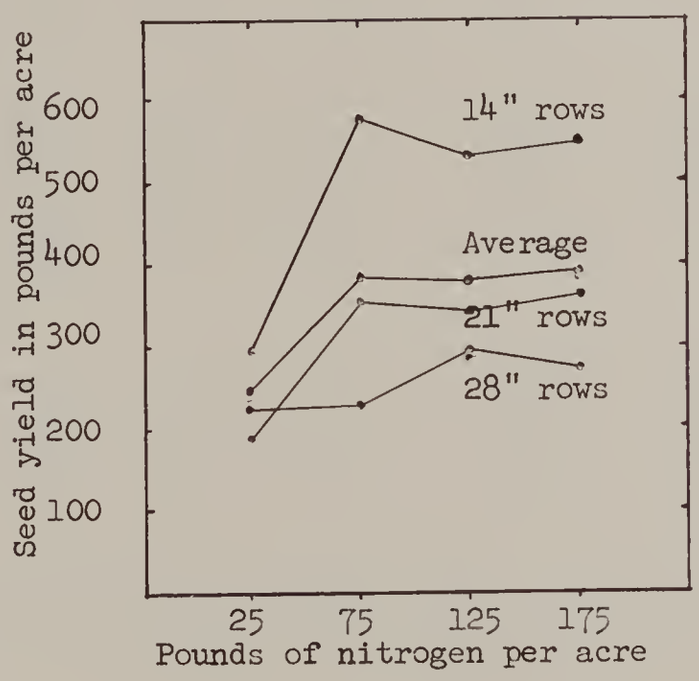
Cultural treatments		Seed		Oil constituents		
Row width	Plants/ft. of row	Moisture	Oil	Epoxy oxygen	FFA	Saponifiables
in.		%	%	%	%	%
12	4	4.59	30.7	3.84	1.04	92.0
	8	4.38	29.4	3.83	1.17	92.2
	16	4.82	28.0	3.80	1.29	92.0
18	4	4.50	29.3	3.82	1.22	92.5
	8	4.63	28.9	3.80	1.16	91.8
	16	4.58	29.4	3.80	1.10	91.8
36	4	4.49	30.5	3.80	1.14	93.0
	8	4.54	30.0	3.82	1.08	92.9
	16	4.53	29.0	3.82	1.08	93.0
Average		4.56	29.5	3.81	1.14	92.4

Georgia - Savannah

A replicated test with three spacings and four nitrogen levels was planted on April 25. Nematodes (*Meloidogyne javanica*) caused considerable damage over much of the experimental area. Harvesting was started earlier than normal because of this damage. Seeds were hand-picked as they matured.

The overall average yield was 351 pounds but the highest individual plot yield exceeded 1100 pounds per acre. The yield from the 14-inch spacing (490 pounds) was significantly greater than from the 21 (309 pounds) and 28 (254 pounds) inch spacings. Seed yields for each spacing increased when 75 pounds of nitrogen was applied as compared to 25 pounds, but there was no advantage in using still higher levels (see graph).

Analytical values were not overly affected by nitrogen levels or row widths. The seed was of fairly good quality. Average analytical values were as follows: seed moisture - 6.20%; oil content - 27.7%; epoxy oxygen - 3.73%; free fatty acids - 2.50%; and saponifiables - 91.1%.



Indiana - Lafayette

Stands in an experiment which involved two row spacings, two planting dates, and two fertility levels were thinned to four plants per foot of row. Days from planting to emergence and bloom tended to be less in the later planting but plant height and seed yield were not affected appreciably. Loss of seed by differential maturation was estimated at 15 to 20 per cent.

Nitrogen level	Row width	Seed- yield	Seed- moisture	Seed- oil	Oil constituents Epoxy oxygen	Oil constituents FFA	Oil constituents Saponifiables
lbs/a	in.	lbs/a	%	%	%	%	%
Planted May 8, 1963							
0	14	760	5.64	25.4	3.61	3.05	93.1
0	38	958	5.65	25.5	3.61	2.93	92.7
50	14	1042	5.69	25.4	3.64	2.50	93.6
50	38	963	5.64	25.9	3.70	2.30	93.1
Planted May 28, 1963							
0	14	1000	5.59	26.7	3.61	2.46	93.5
0	38	1013	5.57	27.4	3.68	2.62	93.5
50	14	872	5.66	27.2	3.65	2.45	92.4
50	38	1049	5.70	27.1	3.66	3.04	92.4

Nitrogen and row width had little effect on the analytical results but the oil content of seed from the May 28 planting was higher than from the early planting.

Another experiment, which consisted of one-half acre of Vernonia in 38 inch rows, was cultivated and combined with regular field equipment. Seed loss of 75 to 80 per cent was partly due to differential maturation but mostly because of unfavorable weather. After a killing frost, it was rainy and foggy thus conditions were unsuitable for combining. A total of 45 pounds of seed was obtained with a combine setting of 1400 RPM cylinder speed and 3 inch clearance. Average analytical values were as follows: seed moisture, 6.20%; oil content, 23.8%; epoxy oxygen in oil, 3.26%; free fatty acids in oil, 11.8% and saponifiables in oil, 86.9%.

Post-frost harvesting does not appear suitable for obtaining high yields of good quality seed.

Iowa - Ames

An early planting was unsuccessful because of cool, damp conditions and a freeze on May 22.

In a May 28 planting, yields from a one foot spacing were usually higher than from two foot rows. A 50-pound application of nitrogen also increased yields. In general, fertilized plants flowered 7 to 9 days later than the unfertilized.

Analytical results show a low oil and a high free fatty acid content. No particular trends were noted as a result of adding nutrients.

Fertility level 1/	Seed yield	Seed-		Oil constituents		
		Moisture	Oil	Epoxy oxygen	FFA	Saponifiables
lbs/a	lbs/a	%	%	%	%	%
		12 inch rows				
0	614	5.16	21.7	3.64	5.16	91.4
N50	1139	5.06	22.1	3.62	4.95	90.6
N50, P25	588	4.99	19.4	3.46	7.38	88.6
N50, P25, K50	710	5.04	21.2	3.56	5.70	89.9
N100, P50	780	5.05	20.7	3.58	5.40	90.8
		24 inch rows				
0	624	5.42	19.9	3.57	5.08	90.8
N50	806	5.14	19.8	3.59	4.90	91.4
N50, P25	873	5.86	20.7	3.60	4.53	92.9
N50, P25, K50	614	7.00	18.4	3.52	5.88	91.4
N100, P50	678	6.67	19.2	3.52	6.22	92.0

1/ Pounds per acre of N, P_2O_5, and K_2O.

A 3.5-acre field was planted to Vernonia with a grain drill in rows 28 inches apart. The field was cultivated with tractor equipment and harvested with a combine. A yield of 220 pounds was obtained. Because of a late frost (October 27), harvesting was delayed until November 4. Stems, which still contained considerable moisture, were tough and difficult to handle with a combine. Earlier harvesting probably would have been preferable.

Localized areas of disease were present in the large field. Diseases were identified as bacterial and Fusarium root rots. Some powdery mildew was also observed.

Kansas- Manhattan

Cultural treatments			Seed yield	Seed-		Oil constituents		
Planting date	Row width	Harvest date		Moisture	Oil	Epoxy oxygen	FFA	Saponifiables
	in.		lbs/a	%	%	%	%	%
			PI 283729 (Pakistan)					
5-3	40	9-30	82	5.15	21.3	3.70	5.70	87.5
5-3	20	9-30	64	5.16	20.5	3.50	4.70	87.6
5-3	40	10-11	50	5.32	14.4	3.35	6.56	84.2
5-3	20	10-11	91	5.32	13.7	3.33	6.35	85.5
5-28	40	9-30	156	4.99	15.6	3.59	5.40	86.0
5-28	20	9-30	220	5.34	18.2	3.63	4.41	86.8
5-28	40	10-11	159	5.85	16.8	3.37	7.53	85.5
5-28	20	10-11	194	5.28	16.3	3.37	7.65	85.1
Bulk sample				5.55	15.1	3.32	6.36	86.2
			PI 263368 (India)					
5-3	40	9-30	139	5.11	19.7	3.68	5.00	88.2
5-3	20	9-30	335	5.09	18.1	3.49	4.41	87.5
5-3	40	10-11	154	5.46	16.0	3.35	7.68	85.7
5-3	20	10-11	92	4.96	15.5	3.56	5.01	87.8
Bulk sample				5.04	15.3	3.34	6.40	86.7
Mixed sample (PI 283729 and 263368)				4.76	15.4	3.54	5.41	87.8

Both Vernonia introductions made excellent early growth but insufficient moisture apparently limited seed yields. The later planting date resulted in higher yields for PI 283729 but harvest date had little influence on yield. PI 263368 outyielded PI 283729 at the early planting date.

All seed samples were low in oil and saponifiables and high in free fatty acids. In general, better quality seed was obtained from the early harvest.

Kentucky - Lexington

A test that was designed to evaluate two row widths and three within-row densities was seeded on May 11. Since Vernonia plants developed slowly and lambsquarter was bothersome, thinning to the desired density was not done.

Plots were harvested by hand on September 20 and 21, and plants were tied in bundles and placed in a greenhouse to dry. Material from border rows was cut, shocked, field-dried and threshed.

Row width	Seed yield[1/]	Moisture	Oil	Epoxy oxygen	FFA	Saponifiables
in.	lbs/a	%	%	%	%	%
Seed which shattered within 10 days after harvest						
14	971	4.88	29.0	3.89	1.88	92.3
21	905	4.95	28.5	3.88	2.04	92.2
Non-shattering seed obtained by threshing (after 10 days curing)						
14	274	5.39	24.4	3.81	2.08	92.3
21	294	5.11	24.0	3.81	2.23	91.8
Field-cured from border rows						
		4.84	27.0	3.85	2.13	92.8

1/ Mean yields for 14 and 21 inch row spacings were 1245 and 1199 pounds per acre, respectively.

The most notable difference in this data is the lower oil content of seed that was obtained by the threshing as compared to that which shattered. One might logically expect more immature seed in the threshed portion, thus a low oil content. However, a change in the epoxy oxygen and free fatty acid content would also be anticipated.

In an unreplicated test, Vernonia was planted at approximately weekly intervals in 21-inch rows on 9 dates; and each planting was divided so that yield determinations could be made for three harvest dates (September 18, 24, and 30).

As shown in the table below, the highest seed yields were obtained from plantings that were made on May 19 and 26.

Oil content was not affected by planting or harvesting dates until the May 19 planting. For this planting, the oil percentages for the three harvest dates were 26.2, 28.9, and 30.3 percent respectively. A similar trend, although lower, was evident for each succeeding planting. In general, free fatty acid content increased with delayed planting. On the other hand, planting and harvesting dates did not cause the epoxy oxygen content to vary appreciably.

Planting date	Days to bloom	Plant height (Sept. 18)	Seed yields			Seed[1]		Oil constituents[1]		
			Sept.18	Sept.24	Sept.30	Moisture	Oil	Epoxy oxygen	FFA	Saponifiables
		in.	lbs/a	lbs/a	lbs/a	%	%	%	%	%
April 22	64	54	480	612	132	5.17	29.9	3.85	2.09	91.6
28	59	57	444	744	580	4.92	30.3	3.89	1.75	92.4
May 5	57	60	840	812	676	5.00	30.2	3.83	2.11	92.0
12	54	63	652	780	808	4.94	31.0	3.83	2.11	91.8
19	59	63	1056	744	1092	4.81	31.3	3.83	2.03	92.2
26	69	61	1296	980	948	4.85	28.5	3.84	2.38	91.5
June 3	61	60	884	804	792	5.02	27.1	3.80	2.92	92.3
10	63	59	912	904	768	5.14	26.5	3.85	2.73	91.6
17	69	49	524	716	692	4.96	24.4	3.75	2.77	92.0

[1] These values are averages from three samples each of which represents a different harvest date.

Maryland - Glenn Dale

Two accessions, PIs 283729 (Pakistan) and 263368 (India, 1961 Texas increase) were evaluated in respect to planting and harvesting dates and row spacings. For harvesting, plants were cut, air-dried under shelter, and hand-threshed. Tabular yield values are presented below.

Date		PI 283729		PI 263368	
Planted	Harvested	30" rows	15"rows	30" rows	15"rows
		lbs/a	lbs/a	lbs/a	lbs/a
4-18	8-14	757	1180	288	240
	8-29	1005	1154	391	395
5-20	9-18	1413	1563	653	737
	10-2	1644	1848	1016	1062
6-11	10-15	1883	1778	995	1020
	10-25	1691	2136	1233	1237
Average		1399	1610	763	782

Results were as follows:

Planting dates - Plants emerged in 20, 10, and 7 to 10 days respectively for April 18, May 20, and June 11 plantings. Apparently there is no advantage in planting before the soil warms up in the spring. Seed yields did increase with delayed planting but harvesting dates were not the same for each planting date.

Row widths - Seed yields of 283729 were higher with 15 inch rows than with 30; but row widths did not influence yields of 263368. Low plant densities of 263368 probably contributed to low yields however Vernonia plants do tend to spread out and compensate for poor stands.

Harvesting dates - The harvesting dates for the first planting were too early for both accessions but especially so for 263368. The latter accession is late-maturing. In most instances, the late harvest for each planting date was more productive than the earlier date.

Oil percentage - In both spacings, the second planting date resulted in the best oil percentage (see table below). The oil content of 283729 was greater than 263368.

Date		PI 283729		PI 263368	
Planted	Harvested	30" rows	15" rows	30" rows	15" rows
		%	%	%	%
4-18	8-14	22.3	24.5	15.6	17.9
	8-29	25.2	28.6	18.4	20.2
5-20	9-18	26.2	27.5	21.9	22.0
	10-2	26.6	27.9	20.4	23.9
6-11	10-15	23.9	21.1	16.8	19.7
	10-25	27.1	21.3	20.2	23.3
Average		25.2	25.2	18.9	21.2

Seed yields were converted to oil yields in pounds per acre as follows:

Planting date	Row spacing	
	15"	30"
4-18	165	124
5-20	330	281
6-11	330	319

A single plot yield of 2415 pounds per acre (283729) indicates that, potentially, oil yields of 400 to 500 pounds are possible.

Epoxy oxygen - The epoxy oxygen content at approximately 3.8% was within the desired range of 3.8 to 4.0%.

Free fatty acids - Generally, the percentage of free fatty acids was somewhat above 2%. The percentage for 283729 was a little lower than 263368.

Nebraska - Lincoln

Plantings of Vernonia included date of planting - row spacing, nitrogen fertilization, herbicide, selection evaluation, and time of harvest studies. Each is discussed separately.

May and June were warm and dry but reasonably good stands were obtained in most seedings. A heavy rain in June caused some stand damage. September and October were quite dry and strong winds before and during harvest resulted in heavy seed losses.

Date of Planting - row spacing study - Seedings were made on three dates in 14; 21; and 28-inch rows. Hand weeding was necessary for good stand establishment. For yield determinations, plants were cut, shocked, and threshed when dry.

Stands from the April 25 planting were so poor that no yields were taken. There were no significant differences in yields between the May and June dates and row spacings.

Date of seeding	Row width	Seed yield	Seed[1/]		Oil constituents[1/]	
			Moisture	Oil	Epoxy oxygen	Free fatty acids
	in.	lbs/a	%	%	%	%
4-25		Poor stands				
5-20	14	313	4.73	22.8	3.57	4.90
	21	255	4.96	20.6	3.56	4.70
	28	362	4.76	20.4	3.56	5.05
6-7	14	361	4.70	23.2	3.45	6.83
	21	361	4.85	21.2	3.48	6.14
	28	357	4.80	19.7	3.40	7.22

1/ These values are averages that were obtained from a low and high seeding rate for each date and spacing.

Nitrogen fertilization - Plants on fertilized plots were greener in color, taller, and late-blooming than the unfertilized check. Differences between treatments were not significantly different but yields were low and variable because of gusty winds and threshing losses. The control yield was the highest.

Nitrogen level	Days to bloom[1]	Seed			Oil constituents	
		Yield	Moisture	Oil	Epoxy oxygen	Free fatty acids
lbs/a		lbs/a	%	%	%	%
0	82	355	4.77	26.6	3.64	6.00
30	86	281	4.91	24.8	3.56	6.30
60	89	330	4.91	24.0	3.53	5.76
90	91	261	5.40	23.9	3.63	6.19

1/ Seeded on June 3 with emergence in five days.

Oil content decreased with increased rates of nitrogen whereas other constituents were largely unaffected. As would be expected, the seed moisture content increased as nitrogen rates increased.

Herbicide test - This pre-emergence weed trial was abandoned because of poor stands. Some of the herbicides appeared promising and a 1964 test at this location is anticipated. Chemical weed control will be necessary if narrow rows are used.

Selection evaluation - Rows of 125 selections (from 1962) were observed. Some were quite uniform while others varied. Several types, such as early maturing, short and late-maturing, and tall-growing, were obtained. Additional selections were made from 1963 plantings.

Time of harvest - A five-acre seed increase field was divided into several sections and harvested at different times. Findings are shown in the following table:

Date		Seed			Oil constituents	
Cut & windrowed	Combined	Yield	Moisture	Oil	Epoxy oxygen	FFA
10-2	10-7	109	4.90	18.0	3.47	6.30
10-7	10-11	216	4.82	19.8	3.40	7.61
10-7	10-14	245	4.97	25.0	3.50	6.02
10-11	10-14	399	4.72	22.6	3.33	10.92
10-15	10-18	292	4.36	22.9	3.40	9.38

Generally speaking, the longer the harvest was delayed, the higher the yields were. Strong winds on October 14 shattered much seed on the uncut portion thus yields from the October 18 harvest were less than from the preceding date. The oil content was higher for the October 7 cutting date (harvested October 14) than for other dates. Epoxy oxygen content was low, and free fatty acid content was high.

The lateness of these cutting and harvesting dates illustrate vividly that earlier maturing, more determinate lines of Vernonia are needed. Actually, the warm, dry conditions in October were favorable for maturing the crop and drying cut plants yet there was much immature seed, and moisture in the stems complicated the combining operation.

North Carolina - Plymouth

Although results have not been reported, experimental plot yields averaged about 800 pounds per acre. A large increase planting was grown at another location.

Oklahoma - Stillwater

Vernonia (1.6 acres) was sown in 40 inch rows and irrigated. Disease symptoms were observed throughout the planting but relatively few plants were affected. Pathologists at Oklahoma State University identified Rhizoctonia in root tissue.

Values in the table are averages from three samples. In general, the chemical characteristics of these samples were fairly good. Epoxy oxygen content was somewhat low while free fatty acids were present in higher quantity than desired.

Date of-		Seed	Seed		Oil constituents		
cutting	threshing	yield	moisture	oil	Epoxy oxygen	FFA	Saponifiables
		lbs/a	%	%	%	%	%
9-3	10-5	370	5.34	27.2	3.71	2.30	90.8
10-5	10-5	628	5.26	24.9	3.61	3.07	90.6
Average		499	5.30	26.1	3.66	2.68	90.7

Pennsylvania - Landisville

Twelve inch rows were used in this study. Rainfall was low therefore irrigated plants averaged about 10 inches taller than non-irrigated ones.

For some reason, seed quality was quite poor. Factors other than moisture were involved since there was no appreciable difference between irrigated and non-irrigated material.

Supp. water	Plants/ft. of row	Seed yield[1]	Moisture	Oil	Epoxy oxygen	FFA	Saponifiables
		lbs/a	%	%	%	%	%
none	4	934	4.61	15.9	2.54	28.1	
	6	670	4.72	17.7	1.96	28.5	
	11	1206	5.75	16.7	2.43	25.5	
irrigated	4	1702	4.59	17.3	2.56	29.1	
	6	782	5.55	14.8	3.24	33.7	62.5
	11	1042	5.59	14.9	2.41	31.8	

1/ More shattering occurred at the intermediate density than others because of later harvesting.

Other locations

Vernonia plantings were made at several locations in addition to those already described. Results from these plantings are presented in the following table. Observational plots were established at other locations but seed samples were not submitted for analyses.

Location	Row width	Harvest method	Seed yield	Seed moisture	Seed oil	Epoxy oxygen	FFA	Saponifiables
	in.		lbs/a	%	%	%	%	%
Arkansas, Fayetteville	28		200	4.54	13.9	3.58	2.75	89.0
Illinois, Dekalb				4.88	22.3	2.94	18.1	
Ohio, Salem	24	hand		6.00	13.9	3.42	9.27	87.4
	24	hand		5.60	13.0	3.20	11.5	83.2
Oregon, Corvallis	30	machine threshed		4.99	25.9	3.78	4.28	91.2
Medford	30	hand-picked	1024	4.89	29.0	3.86	1.36	92.2
	12	cut, dried, & threshed	634	6.05	25.3	3.74	2.80	92.2
Ontario	12	hand-picked as mature		6.09	28.9	3.72	3.82	91.0
	12	cut and dried		6.32	20.7	3.69	5.36	89.8
Pendleton	24	cut, dried and threshed	94	7.67	19.6	3.24	15.0	
Minnesota, Rosemount	36	windrow dried	190	4.66	26.8	3.51	8.73	86.7
	36	artifically dried	280	4.74	27.3	3.61	6.70	88.8
Mississippi, Popularville	48	hand	164	6.09	22.8	3.47	3.48	92.7
North Carolina 1/	42	cut and shaken	500	5.35	26.5	3.81	1.26	92.7
Pennsylvania, Univ. Park	12			6.34	13.7	2.24	29.2	
South Carolina, Clemson	36	cut and theshed by hand	217	4.81	27.1	3.68	2.66	91.9
Texas, College Station 2/	40	plant selection		4.43	21.0	2.86	2.81	91.6
	40	early harvest (hand)		5.50	22.3	2.43	4.97	79.6
	40	late harvest (hand)		5.24	22.3	2.80	4.71	90.2
Wyoming, Cheyenne 3/				5.99	24.5	3.55	1.91	93.8

1/ Average of 3 samples

2/ PI 263368

3/ Only one sample was submitted for analysis but yields were 1132, 1014, 929, and 391 pounds per acre for row widths of 7, 12, 14, and 20 inches, respectively.

DISCUSSION AND SUMMARY

Considerable experience in growing this indeterminately-flowering member of the Compositae family was gained during the 1963 season. While many of the findings were discouraging, it is apparent that Vernonia does have a good yield potential. Further, even though only a few accessions are available, heterogeneity in maturity, plant stature, and other characteristics indicates a broad base for selection activity.

Efforts in evaluating and making selections should be accelerated in 1964 possibly even to the extent of making small greenhouse seed increases during the fall and winter months. Some suggested selection objectives are:

1. More uniform flowering types
2. Earlier maturing lines especially for central and northern locations
3. Shatter resistance
4. Plants shorter in height (of value from harvesting and lodging aspect)
5. Disease and nematode resistance
6. Lack of seed pappus

Insects have not been bothersome but damage from root rots, wilt, and root-knot nematodes was evident. Also, symptoms indicative of virus infection were noted.

Seedlings emerge more rapidly if planting is delayed until warm weather. Quick stand establishment should lessen weed competition. The number of days from planting to bloom is greater for early plantings as compared to later ones. Seed from late plantings does, however, tend to be lower in oil than from earlier dates. To enumerate approximate planting dates, results indicate that planting in mid-May is best for Kentucky, but late May is more suitable for Indiana, Iowa and Nebraska. These dates would vary each year according to local conditions.

In most experiments, the highest yields were obtained from narrow rows. But Vernonia is not vigorous enough to effectively compete with weeds during early stages of growth. Therefore, if narrow rows are to be used, chemical weed control will be required. Row width and within-row density have a moderate effect on the amount of seed oil and its constituents.

Seed yield response to nitrogen, phosphorous, or potassium applications was not clearcut in 1963. Nitrogen delayed maturity somewhat and resulted in increased yields in some instances.

Many experimental plots were harvested entirely by hand but mechanical means will be necessary for large experimental or commerical plantings. In this connection, timing is a critical factor in relation to obtaining maximum yields with good seed quality and avoiding unfavorable weather conditions. Allowing plants to be killed by frost before direct combining does not appear to be a good practice because weather poses more of a hazard, and heavy seed losses occur by delaying harvest. Cutter-bar contact with plants also causes severe shattering. Seed obtained in this manner has been of poor chemical quality with low germination. At Nebraska, windrowing and subsequent combining were successful but yields were low, and the seed oil was high in free fatty acids. Large plants dry slowly and are difficult to run through harvesters. From the foregoing discussion, the need for small plants that mature earlier is emphasized.

The possibility of multiple-harvests with specialized grass, flail, vacuum, or other type harvesters should not be overlooked. Plants would likely be damaged considerably by the harvesting operations but the seed should be of good quality and more seed would ripen on the injured plants. This approach would necessitate the use of wide rows.

Chemical defoliation with direct combining is another possible harvesting method but some seed loss due to cutter-bar contact would be expected.

Swathing or windrowing preferably should be done when dew is present, thus seed shattering is minimized.

Domestically produced seed has germinated slowly and poorly. In contrast, the Pakistanian seed (PI 283729) germinated very well. It is likely that this seed was hand-picked as mature and stored at air temperatures under cover. Cold storage was probably not involved until the seed was received in the United States. Freshly harvested seed from plots does not germinate well regardless of the chemical quality. A period of over-ripening may be required however drying seems to be an important and, perhaps related, factor. Some preliminary work along this line was done at Glenn Dale and more detailed studies are anticipated later.

In summation, 1963 Vernonia results from 26 locations (20 states) are reported herein. Specific mention of observation plots at other locations was not made. After considering the results, which include a discussion of the relationship between cultural treatments and analytical values, the need for selection of better plant types is evident. Other areas of research that require attention in 1964 include weed control and harvesting studies.

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UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
Crops Research Division
New Crops Research Branch
Beltsville, Maryland

ANNUAL PULP CROPS - 1963 FIELD EXPERIMENTATION WITH KENAF AND SUNN HEMP

George A. White and J R. Haun - Chemurgic Crop Investigations

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INTRODUCTION

This report summarizes 1963 field experimentation with kenaf (Hibiscus cannabinus) and sunn hemp (Crotalaria juncea). Results from the evaluation of certain Sesbania and okra introductions or selections in the Southern region are also included. This summary is intended to serve as a guide for future research activity with these potential annual sources of pulp.

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TRIALS BY LOCATION

Texas - College Station

Two pulp trials, each with 40-inch rows, were tractor cultivated but no supplementary water was added. In the regional trial, the *Sesbania* and okra entries yielded so poorly that further evaluation does not seem necessary. Generally, yields of all entries were low. Row spacings of 20 inches or less appear desirable for obtaining high yields.

The moisture levels at harvest for the Crotalarias and Everglades 41 would be low enough for storage provided the material was unchopped.

Crop	Dry-weight yield	Plant height	Plants/ foot of row	Moisture at harvest
	lbs/a	ft.		%
Crotalaria juncea (Line 374)	5889	8	5.8	29.4
C. juncea (PI 248491)	7001	10	5.8	22.2
Okra (PI 120833)	2207	4	3.8	50.0
Sesbania arabica (PI 167069)	3162	8	5.0	37.5
Sesbania (Okla. No. 42)	2616	9	6.0	50.0
S. cannabina (PI 180050)	4021	9	4.2	40.0
Kenaf - Everglades 71	6442	9	7.2	37.5
Everglades 41	8829	10	7.2	25.0

Yields of Crotalaria and kenaf that were obtained from the second experiment did not differ greatly (see below).

Crop	Dry-weight yield	Plant height	Stem diameter	Moisture at harvest
	lbs/a	ft.	mm	%
Kenaf - Everglades 71	6409	9	19	38.8
Everglades 41	7063	10	21	40.0
Crotalaria juncea (Line 374)	6984	9	14	33.3
C. juncea (PI 248491)	7099	10	13	27.7

South Carolina

Not reported

Puerto Rico

A seed increase of C. juncea, PI 248491, was made at the Isabella Station. Rows were spaced 30 inches apart and within-row densities varied from 1 to 4 plants per foot of row. Harvesting began on March 25.

Oklahoma - Stillwater

Crop	20-inch rows			40-inch rows		
	Dry-weight yield	Height	Plants/ft. of row	Dry-weight yield	Height	Plants/ft. of row
	lbs/a	in.		lbs/a	in.	
Okra selection	2512	49	1.1	3412	62	1.2
Okra (120833)		no stand				no stand
C. juncea (L.374)	7087	42	2.7	4259	96	2.6
C. juncea (248491)	6816	90	1.2	4663	136	1.6
Sesbania selection	2699	124	2.6	2435	124	2.6
Sesbania (180050)	2748	128	1.9	2838	136	2.0
Kenaf (E. 71)	7253	104	1.7	8021	100	1.8
Kenaf (E. 41)	6216	110	2.2	8128	116	2.5
LSD .05	280			281		
.01	392			394		

This test was planted on May 17. Total rainfall from May 1 through October was 19.84 inches; but distribution apparently was not favorable to growth of the pulp plants. The average rainfall for this period is approximately 21.18 inches.

The okra and Sesbania entries were not promising at either spacing. Sunn hemp produced better yields when grown in 20-inch as compared to wider rows but the opposite was true for kenaf. Thicker stands of the Brazilian sunn hemp would be preferable.

North Carolina

Yield results from two locations were as follows:

Crop	Dry-weight yield Plymouth	Dry-weight yield Willard
	lbs/a	lbs/a
Kenaf (E. 41)	8400	13200
Kenaf (E. 71)	10200	13200
Sunn hemp (L. 374)	5600	6000
Sunn hemp (248491)	7200	8000

Kentucky - Lexington

A replicated kenaf planting on a Captina silt loam was seeded on May 11. Monthly rainfall distribution was good throughout the season except during September and October however temperatures were somewhat below average until October. The first killing frost occurred on October 29. Since yield samples were removed on November 8, considerable leaves were retained and the dry-matter percentage was low (about 32%). Results are shown below.

Row width	Seeding rate*	Plant height	Stem diameter	Dry-weight yield Whole plants	Dry-weight yield Stems only	Plants/ sq. ft.
in.		in.	mm.	lbs/a	lbs/a	
7	low	96	13.0	12927	10688	3.4
7	high	93	11.2	12897	10446	5.0
14	low	100	14.0	11370	9152	1.9
14	high	97	14.2	11971	9718	2.9
42		110	24.2	9533	7045	.5

* Seeding rates were 6 (low) and 9 (high) seeds per foot of row.

Georgia - Experiment

Not reported

Florida - Belle Glade

Each year, kenaf seed for experimental pulp trials is increased at this location. Kenaf varietal trials were set up cooperatively between the Belle Glade Station and certain State Stations. Results are not included in this report.

Florida - Gainesville

Several kenaf accessions were grown in unreplicated tests on two soils in 38-inch rows. Although yields were generally low, certain introductions appeared promising (see below).

Plant Introduction Number	Dry-matter yields	
	Gainesville sandy loam	Leon fine sand
	lbs/a	lbs/a
248895	12869	
250037	2313	11627
267667	16393	
196988	18456	7314
196989	5473	10359
190126	5190	10579
190127	10070	5483

In the regional cooperative test, yields varied greatly between replications, hence average yields were low. Everglades 71 yielded approximately 11.5 tons of dry matter in one replication but was quite poor in others. Nevertheless, these results indicate a very high yield potential for kenaf. Sunn hemp stands were poor and the Brazilian strain showed evidence of virus infection

Crop	Dry-weight yield	Plant height
	lbs/a	ft.
Kenaf (E. 71)	10662	18
(E. 41)	9078	18
Okra (120833)	not harvested	1.5 to 2
C. juncea (Line 374)	4083	7
C. juncea (248491)	6295	8

Nebraska - Lincoln

Kenaf and sunn hemp experiments were conducted on a Wabash silty clay loam which tested low in nitrogen. Plots were seeded on June 3 and harvested on December 3. On July 23, the experiments were irrigated with about 3 inches of water.

Variety	Row spacing	Nitrogen level 0	50	100	150
	in.	lbs/a			
Everglades 41	14	14460	16080	16420	15820
	21	11920	12460	12660	12360
	28	12120	10820	13560	11060
Everglades 71	14	16140	16080	15720	16960
	21	14040	15480	16080	14540
	28	13800	14700	14520	14420

Statistically, the mean yield of Everglades 71 was significantly greater than that of Everglades 41. Also, significantly higher yields were obtained from the 14-inch row spacing than from 21- and 28-inch spacings.

Dry-matter tonnage increased slightly with nitrogen application but the difference was not significant.

Everglades 41 plants averaged 2 inches taller than Everglades 71 plants. Also, plants grown in 14-inch rows were 2 and 3 inches shorter respectively than plants from 21- and 28-inch rows. These differences were significant. Nitrogen treatments had little effect on plant height.

The average stem diameter of plants grown in 14-inch rows was significantly smaller than that of plants grown in wider rows.

The number of plants per linear foot of row was similar for each variety. Results indicate that stands were not adversely affected by application of high rates of nitrogen.

Emergence of sunn hemp seedlings (about 5 days) was rapid but stands were only fair and weeds were a problem. The over-all average yield was 7600 pounds per acre. The 14-inch row spacing yielded better than either of the wider spacings. There was no significant nitrogen effect; however, all nitrogen treatments resulted in yields that were lower than the check.

Sunn hemp plants tended to be shorter in wider row spacings. This species branches considerably when plant density is low. Crotalaria yields were as follows:

Nitrogen level	Dry-matter yield (lbs/a) 14"	21"	28"	Mean	Statistical significance (5%)
0	10060	7980	7080	8380	a
50	8600	9260	5300	7720	a
100	9880	5880	5080	6940	a
150	9840	6940	5900	7560	a
Mean	9600	7520	5840	7660	
Stat. sign. (5%)	a	a b	b		

Minnesota - Rosemount

Crop	Row width*	Seeds sown/ sq. ft.	Plant height	Stems/ sq. ft.	Dry-weight yields
	in.		in.		lbs/a
Kenaf	6	12	45	3.0	2722
Everglades 71	12	6	62	4.0	9801
	24	6	62	3.7	8903
	40	6	66	2.6	8698
	40	3.6	70	2.3	8764
Kenaf	6	12	50	8.9	6207
Everglades 41	12	6	64	2.8	7187
	24	6	67	3.4	9379
	40	6	70	2.2	8870
	40	3.6	70	1.5	7334
C. juncea	6	12	56	2.6	1198
(248491)	6	24	66	3.3	1089

* 6 inch spacings not cultivated. Others cultivated and weeded.

Crotalaria juncea does not appear adapted to Minnesota conditions. Also, field cultivation or chemical weed control seemingly will be necessary for successful kenaf production.

Kansas - Manhattan

Excellent kenaf and Crotalaria yields were obtained from a planting (12-inch row width) on a Sarpy, fine sandy loam. Approximately one inch of water was added on August 6; but, since a 1.5-inch rain fell during the week of August 16, yields would have been good even though no water was added.

Kenaf responded to nitrogen application and the 50 pound rate was best for the early planting, whereas 100 pounds was superior for the later date. Crotalaria yields were also increased by adding nitrogen. The May 17 planting date was preferable for kenaf.

These annual pulp crops continue to look promising in Kansas.

Crop	Nitrogen level	Planted May 3		Planted May 17	
		Dry-weight yield	Plant height	Dry-weight yield	Plant height
		lbs/a	ft.	lbs/a	ft.
Kenaf	0	13040	8.1	12920	9.8
Everglades 71	50	16100	8.8	18380	11.2
	100	13400	8.4	21200	11.0
Kenaf	0	18340	8.8	16540	10.7
Everglades 41	50	23740	10.3	24080	11.5
	100	21280	9.8	28480	11.0
C. juncea	0	18300	11.8	17540	10.2
(248491)	50	18540	11.8	19400	10.5
	100	21120	11.8	19280	10.6

Iowa - Ames

Fertility trials with kenaf and Crotalaria (unreplicated) included two planting dates, May 8 and 28. The early planting was severely set back by a freeze on May 22. In addition, unfavorable soil conditions (perhaps alkalinity or poor drainage) restricted plant development. Many kenaf plants were yellow and stunted thus only a few plots were harvested. Crotalaria yields, while not high, were not affected as much as those for kenaf by the adverse soil conditions.

Both Everglades 71 and 41 yielded about 7 tons per acre in some of the better plots.

No Crotalaria yield samples were taken from the early planting. Some representative yields from the May 28 planting were as follows:

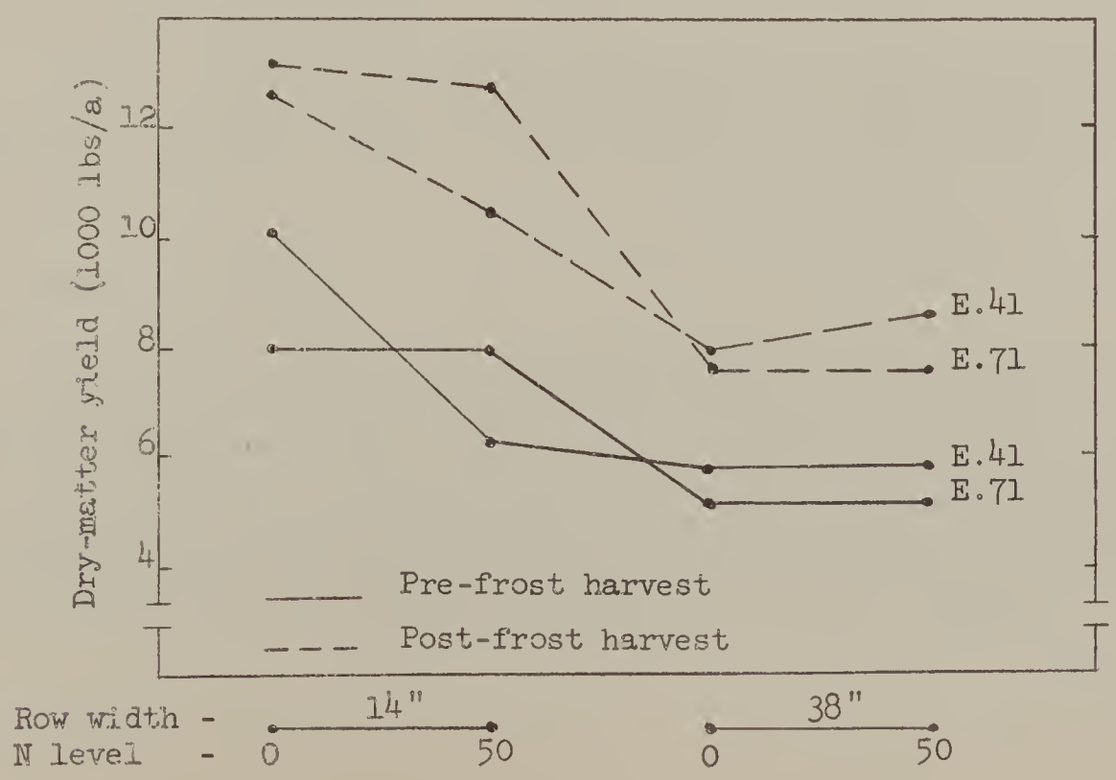
Fertility level*	Dry-weight yield
	lbs/a
0	8131
N50	8712
N50, P25	9293
N50, P50	9002
N100, P50, K50	4356

* Pounds of N, P_2O_5, and K_2O per acre.

Indiana - Lafayette

Two kenaf varieties were planted (May 10) on a Chalmers silty clay loam. A split-plot arrangement was set up to evaluate two nitrogen levels and two row spacings. Plants were damaged by frost on May 22 and a severe wind storm on June 10. Stands averaged 7 to 13 plants per foot of row. Two harvests, pre- and post-frost, were made.

Yield results are shown graphically. The highest yields were obtained from plots with 14-inch rows with no nitrogen. Spacing had little effect on stalk diameters, but plants in wide rows did branch more. In general, plants did not look good and appeared stunted. Early weather conditions undoubtedly reduced yields.



Pennsylvania - Landisville

Plantings of kenaf and sunn hemp were made on a Hagerstown silt loam. Seeding date was May 7. Stands were thinned to 4 plants per foot of row. A freeze occurred on May 24.

Crop	Irrigation	Plant height	Stem diameter	Dry-weight yield
		in.	in.	lbs/a
Kenaf (E. 71)	+	49	9/16	4323
	-	34	10/16	2493
Sunn hemp	+	63	7/16	5772
(248491)	-	38	4/16	3310

As shown above, yields were quite low but temperature probably was the most important factor that contributed to these results. These species often do not fully recover from being subjected to a low temperature such as was experienced here.

The May 24 freeze was not typical however a late May planting date might be more appropriate for this location.

Pennsylvania - Huntingdon

A June 17 planting of kenaf and sunn hemp was made on bottom land. Rows were 12 inches apart. Dry-weight yields were not obtained. Sunn hemp plants averaged about 11 inches taller than kenaf plants but they emerged unevenly.

Maryland - Glenn Dale

Studies are continuing on the development of a new method for measuring plant response to environmental factors. By closely following leaf development, changes in the growth rate can be detected. Then, through the use of multiple correlations, an equation, which expresses the relationship of the environmental factors and plant growth, is formulated. The equation can be used to predict growth or identify needed environmental changes (such as soil moisture). Kenaf, and certain other new crop species are the test plants for these studies.

A 1963 test with kenaf, which compared scheduled (based on leaf development) and regular (based on gross observations) irrigation, showed a decided advantage for the former method.

Planting date	Plant height	Stems/ft.^{2}	Dry-matter yield
	ft.		lbs/a
		Scheduled irrigation	
4-16	9.8	1.69	13239
5-20	9.1	1.78	13002
		Regular irrigation	
4-16	8.4	1.89	9634
5-20	8.2	1.98	10140

Poor development of kenaf and sunn hemp plants in an irrigated experiment (18-inch rows) resulted in low yields and severe weed competition. Stands of sunn hemp were poor and several plants died prematurely but the cause was not determined. Virus infection was noted, however. In contrast, a good stand and vigorous growth characterized another nearby sunn hemp planting but no yields were taken.

DISCUSSION AND SUMMARY

Excellent yields of kenaf and sunn hemp were obtained at the Nebraska and Kansas stations in 1963. Unfavorable soil and weather conditions contributed to low yields in Iowa, Indiana, and Pennsylvania. Soil moisture was not adequate in Texas and Oklahoma.

Kenaf is more promising as a potential pulp crop than sunn hemp. In many cases, the leguminous sunn hemp plant produces low yields because of inadequate stands. As a result of this inadequacy, weed competition may become severe. A somewhat greater plant population for sunn hemp as compared to that for kenaf will probably be required. When sunn hemp seed is of poor quality, seeding rates should be adjusted accordingly.

It is unlikely that profitable yields can be produced unless row-widths of 20 inches or less are used. Generally, 14-inch rows are recommended but yields from Kentucky were greater for 7-inch rows. Similar results

were obtained in North Carolina from the 1962 season. Rows wide enough to allow tractor cultivation (20-inch) were very satisfactory in Texas (1962). In general, humid areas with relatively short growing seasons require high plant densities and narrow rows whereas, in dryer areas, less dense stands are satisfactory.

Both pulp species responded to nitrogen application in Kansas, but responses were not clear-cut at other locations. Even though the experimental plot soil tested low in nitrogen, kenaf yields were not significantly improved by nitrogen application in Nebraska tests. Since kenaf and sunn hemp are sensitive to cool temperatures, fertility effects could be masked or erratic.

Future activity with these species should be directed toward improving yields and toward determining suitable harvesting and handling methods. Although field evaluation of other species and of new kenaf and *C. juncea* introductions is desirable, this work should not supersede advanced evaluation of the potentially high-yielding varieties and strains that are now available. Okra is of special interest because its woody fibers are longer than those of kenaf; but we have no varieties or selections that are comparable to kenaf in productivity.

* *

UNITED STATES DEPARTMENT OF AGRICULTURE
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AGRONOMIC EVALUATION OF CERTAIN SORGHUMS AS PULP SOURCES[1/]

Compiled by

George A. White and J. R. Haun

Chemurgic Crop Investigations

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1/ This report summarizes cooperative crops research on sorghums as a source of pulp. Publication, display, or distribution of any data or statement herein should not be made without prior approval of the New Crops Research Branch, CR, ARS, USDA and the cooperating agency or agencies concerned

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INTRODUCTION

The suitability of sorghum for making paper and other pulp products has been known for many years. Concentrated research to this end began within the past decade. Perhaps the most detailed research to date has been done in France.

In the United States, crops research on sorghums for pulp began, for all practical purposes, in 1960. Some agronomic data had been obtained from prior seasons, and this earlier activity was concurrent with the analysis of samples in the Northern Utilization Laboratory screening program. Many of the samples were sent from Chillicothe, Texas; and, as a result, certain sorghums were recommended for further field evaluation in 1963. Until the 1963 season, most of the field research was with Sorghum almum (sorghumgrass or Columbusgrass) because this species looked especially promising as a result of screening evaluations.

It should be kept in mind that crops and utilization research on sorghums for pulp is still in its infancy. Proper cultural, harvesting and handling methods have not been ascertained. In addition, the amount of pith allowable in pulping processes, the contribution of leaves to pulp yield and quality, and other utilization questions remain unanswered. Thus, industrial interest in the sorghums has not developed. It is hoped that, with further agronomic and utilization studies, a use-cost potential can be presented to industries and thereby stimulate interest.

In screening evaluations, seeds are removed because of chemical interference. Leaves and culms are usually processed together but, in some cases, it is necessary to evaluate them separately. Differences in sample preparation may influence the procedures that are used. Regardless of harvesting and handling methods, some leaf material will likely enter into the pulping processes.

1963 FIELD RESULTS

Eight sorghums were recommended for field evaluation. F.C. 36204 (Tift sudan) was substituted for F.C. 34641 because seed of the latter was not available. Also, an additional *S. almum* entry (PI 202410) was included in all tests except at Chillicothe, Texas. Further identification of these sorghums is provided in Table 1.

Table 1 - Identification of sorghums being evaluated as pulp sources

Identifying number 1/	Type	Country of origin	Year of introduction
PI 88000	kaoliang	Korea	1930
PI 88004	kaoliang	Korea	1930
PI 177549	broomcorn	Turkey	1949
PI 179749	durra	India	1949
PI 190579	*Sorghum almum*	Argentina	1950
PI 202410	*S. almum*	Argentina	1952
PI 229837		South Africa	1955
PI 229847		South Africa	1955
FC 36204	Tift sudan		

1/ The letter designations refer to plant introduction and forage crop numbers.

Cultural details for each location are given in Table 2.

Table 2 - Cultural methods used for the 1963 sorghum evaluation tests

Location	Date of planting	Row width	Date of harvest
		inches	
Experiment, Georgia	May 18	36	Sept. 7 Sept. 19
Lafayette, Indiana		38	Pre-frost Post-frost
Ames, Iowa	May 31	12	October 10
Rosemount, Minnesota	June 4	30	November 7
Chillicothe, Texas	July 19	40	November 27
College Station, Texas	April 17	20	August 15

Texas - Chillicothe

The only yield data before 1963 for most of the test entries was obtained from this location (Table 3). Yields from numbers 229837 and 229847 were quite high in 1960 but much lower in 1961. Broomcorn (177549) was the most consistent of all entries over the three year period. Tift sudan appeared promising only in 1960. The kaoliangs did not demonstrate much of a yield potential.

Table 3 - Yields of certain sorghums grown at Chillicothe, Texas, prior to 1963

Identifying number	Type	Air-dry yields 1959	1960	1961
		lbs/a	lbs/a	lbs/a
FC 34641	Tift sudan	6150	12740	5600
PI 88000	kaoliang	7700	9110	4380
PI 88004	kaoliang	7880		
PI 177549	broomcorn	10320	9940	7220
PI 179749	durra	6440	11320	3140
PI 229837			17260	5790
PI 229847			18180	6310

In order to obtain stands in 1963, pre-planting irrigation was necessary. Plots were subsequently irrigated on August 8 and September 5 because summer rainfall was quite low. Budworms damaged stands and may have delayed maturity in some instances. Sorghum midge caused some irregular reduction in grain yields.

Numbers 229847, 229837, and 177549 outyielded other entries; but, considering that yields were determined on an air-dry basis, none of the yields were high (Table 4). The kaoliangs and durra were very low yielding and seem to have little potential at this location.

Table 4 - Sorghum evaluation at Chillicothe, Texas. 1963

Identifying number	Air-dry yields lbs/a	Statistical significance 1/	Seed maturity	Plants/ft. of row	Plant height inches	Stalk diameter cm
PI 229847	9872	a	soft dough	2.6	83	1.3
PI 229837	8953	ab	hard dough	1.9	100	1.6
PI 177549	7610	bc	mature	2.5	89	1.4
CH 61001 2/	6830	cd	mature	8.0	83	.7
FC 36204	6517	cd	mature	10.8	77	.6
PI 190579	6305	cde	mature	7.4	82	.7
PI 179749	5833	de	mature	3.7	66	1.0
PI 88000	4853	ef	mature	3.5	89	1.2
PI 88004	3900	f	mature	2.3	84	1.1

1/ Means followed by the same letter are not significantly different at the 5% level.

2/ Commercial seed of S. almum was substituted for PI 202410 and assigned a Chillicothe (CH) number.

Texas - College Station

Very dry conditions caused rapid maturing and drying of most entries and replicate yields were highly variable. The early maturation probably accounts for the low percentage of culms as compared to leaves in contrast to results from other locations (Table 5). The kaoliangs lodged badly and, along with durra (PI 179749), yielded poorly.

Table 5 - Sorghum evaluation at College Station, Texas. 1963

Identifying number	Dry-weight yields1/	Moisture at harvest	Seed maturity	Yield composition			Plants/ foot of row	Plant height	Culm diameter
				culms	leaves	heads			
	lbs/a	%		%	%	%		inches	mm
PI 202410	13596	40.1	mature	47	36	16	10.3	76	6.8
PI 177549	11217	34.8	mature	46	39	14	4.8	84	9.7
PI 229837	10618	29.7	hard dough	46	44	10	4.1	82	10.4
PI 190579	8936	44.6	mature	46	33	21	7.1	64	7.5
FC 36204	8835	42.2	mature	50	27	22	9.6	70	5.0
PI 229847	8730	24.4	no seed	47	53	0	4.7	60	9.0
PI 88004	7779	49.6	mature	34	30	36	3.5	71	7.1
PI 88000	7385	49.9	mature	34	42	24	5.5	52	5.3
PI 179749	6967	38.1	mature	43	36	21	5.3	68	6.8

1/ The yield for PI 202410 is significantly greater at the 5% level than yields for PIs 88004, 88000, and 179749.

Georgia - Experiment

Good stands were obtained and two cultivations provided satisfactory weed control. Two dates (September 7 and 19) of harvest were used in order to get plants at maturity. Some midge damage to seed formation was noted. Also, considerable lodging occurred with PIs 177549 and 179749 and lesser amounts with PIs 202410 and 190579. Yields and other information are reported in Table 6.

In view of the high percentage of culms, broomcorn (PI 177549) was especially promising even though it lodged rather severely.

Table 6 - Sorghum evaluation at Experiment, Georgia. 1963

Identifying number	Dry-weight yield	Seed maturity	Yield composition[1] culms	leaves	heads	Stalks/ ft. of row	Plant height	Culm diameter	Leaf disease[2]
	lbs/a		%	%	%		feet	inches	
PI 177549	15780	mature	65.8	16.7	17.5	9.2	11.8	.8	3
PI 229847	14000	dough	61.8	26.8	11.4	4.0	11.0	.7	1
PI 190579	13928	mature	56.5	31.6	11.9	7.2	8.0	.3	2
PI 229837	13672	dough	63.2	28.3	8.5	4.3	11.8	.8	2
PI 88000	13448	mature	57.8	30.5	11.8	11.6	9.2	.4	1
PI 88004	12362	mature	57.9	25.3	16.8	6.2	9.2	.4	1
PI 202410	9120	mature	57.5	34.6	7.9	8.0	8.5	.4	2
FC 36204	7922	mature	63.7	27.4	8.9	9.2	7.8	.3	1
PI 179749	6628	mature	53.4	24.6	21.9	4.8	7.0	.4	1

1/ Hand separations were made on pulping samples at Peoria.

2/ Disease rating - 0, no disease; 5, severe disease.

Minnesota - Rosemount

As shown in Table 7, yields at this location were very good. The kaoliangs yielded surprisingly well but the yield composition for PI 88000 was far from desirable.

A killing frost occurred on October 28 and plots were harvested November 7. At this time, all entries contained 45% or more moisture. Lack of good drying weather late in the season may be a limiting factor for production of sorghums or other annual pulp crops in northern areas. It is likely that harvesting methods will have to be modified.

By November 13, PI 229847 was still erect whereas other entries had many broken stalks. PI 88000 lodged badly.

Considering yields and yield composition, PIs 229847, 229837, and 177549 appear the most promising for Minnesota. The first two of these are late, thus little seed would be produced.

Table 7 - Sorghum evaluation at Rosemount, Minnesota. 1963

Identifying number	Dry-weight yield	Statistical significance[1]	Moisture at harvest	Seed maturity	Yield composition[2] stems	leaves	heads	Stalks/ sq.ft.	Plant height	Stalk diameter	Lodging score[3]
	lbs/a		%		%	%	%		inches	inches	
PI 229847	14013	a	46	anthesis to mature	56.5	38.7	4.8	3.7	116	.50	1.0
PI 177549	11082	b	51	hard dough	60.0	23.0	17.1	3.0	127	.50	1.0
PI 88000	10756	b	45	mature	34.5	33.7	31.8	3.1	108	.56	6.0
PI 88004	10749	b	46	mature	56.7	29.0	14.3	3.1	118	.50	1.7
PI 229837	10600	b	58	milk	60.2	33.4	6.4	3.5	118	.56	1.0
PI 202410	9358	b	51	mature	53.6	33.5	12.8	4.1	114	.44	2.0
PI 190579	9332	b	46	mature	50.8	31.3	17.9	4.4	109	.38	2.3
PI 179749	8769	b	55	mature	56.6	19.8	23.6	2.8	88	.38	3.7
FC 36204	6346	c	57	mature	39.2	29.6	31.2	6.8	91	.25	3.7

1/ Means followed by the same letter are not significantly different at the 5% level.

2/ Hand separations were made on pulping samples at Peoria.

3/ Values are based on a scoring of one (erect) to nine (flat).

Iowa - Ames

The nine sorghums were planted in 12-inch rows on a uniformly fertilized area. After one wheel hoeing, all lines competed very well with weeds. Light yellowing early in the season suggested some nitrogen deficiency.

Yields from all lines were exceptionally good (Table 8). Durra

Table 8 - Sorghum yields and composition at Ames, Iowa. 1963

Identifying number	Dry-weight yield	Moisture at harvest	Yield composition 1/			Stalk yield
			culms	leaves	heads	
	lbs/a	%	%	%	%	lbs/a
PI 229837	24451	39	57.0	35.0	8.0	13937
PI 202410	21911	49	56.1	35.5	8.4	12292
PI 190579	21373	50	56.5	32.3	11.2	12076
PI 179749	20850	25	50.3	27.8	21.8	10488
PI 229847	18934	46	66.8	25.1	8.1	12648
FC 36204	18925	24	51.1	28.7	20.2	9349
PI 177549	16451	57	66.3	26.7	7.0	10907
PI 88004	14317	45	62.4	25.0	12.6	8934
PI 88000	13649	52	53.8	28.0	18.2	7343

1/ Hand separations were made on pulping samples at Peoria.

(PI 179749) yielded well but the culm percentage was not very favorable. Low stalk yields were obtained only from FC 36204 (Tift sudan) and the kaoliangs.

Although yield composition was generally very favorable for pulping, it was influenced by seed losses (bird damage) and probable loss of lower leaves because of dry conditions before harvest. Stalk yields would not, of course, be affected by these losses.

Information about lodging, plant size, etc., is given in Table 9.

Table 9 - Sorghum characteristics. Iowa, 1963.

Identifying number	Dough stage of maturity	Plants/foot of row	Plant height inches	Lodging score1/	Rust infection
PI 229837	soft	6	126	0	light
PI 202410	soft	12	108	0	light
PI 190579	soft	11	114	1	heavy
PI 179749	soft	5	96	3	moderate
PI 229847	milk	5	132	0	light
FC 36204	medium	14	102	3	light
PI 177549	soft	8	132	0	light
PI 88004	soft	6	108	1	heavy
PI 88000	soft	7	102	4	light

1/ Values are based on a scoring of zero (erect) to five (flat).

Indiana - Lafayette

Two harvests (pre-frost at hard dough stage and post-frost) were made. There was considerable variation among entries in number of stems harvested per row (Table 10) but most of this variation was due to differences in tillering. Results are very encouraging as the yield potential apparently is quite high.

Table 10 - Sorghum evaluation at Lafayette, Indiana. 1963

Identifying number	Dry-weight yields (pre-frost)		Dry-weight yields (post-frost)		Stems/foot of row	Plant height[1]	Days to maturity
	Total	Stover	Total	Stover			
	lbs/a	lbs/a	lbs/a	lbs/a		ft.	
PI 177549	20200	14600	22800	18000	7.0	10.0	123
PI 229837	19800	18800	21200	19600	5.3	9.4	144
PI 202410	17400	15600	19200	16600	17.1	7.9	124
PI 229847	16600	14200	26600	23400	4.6	8.8	148
PI 88000	16600	10600	15400	12000	9.7	9.9	124
PI 190579	15600	13000	19800	18200	18.5	8.6	109
PI 179749	14600	9800	11400	6600	7.5	7.5	107
PI 88004	14200	9200	13800	11800	6.3	9.6	113
FC 36204	12200	8200	17600	14200	20.2	7.7	117

1/ Height measurements were made on 8-1-63.

EARLIER EXPERIMENTATION WITH SORGHUM ALMUM

Although several samples of S. almum were screened earlier, PI 190579 and later introductions were not evaluated until after the 1960 crop season. Unfortunately, this particular introduction was not grown each season thereafter at cooperating locations because several other lines seemed to have a greater yield potential.

During the period of 1960 through 1962, the cooperating NC-7 states that grew S. almum at least one season include Iowa, Indiana, Kansas, Minnesota, and Missouri. Oven-dry yields for 1962 from three locations are given in Table 11. For the sake of brevity, earlier results are not reported.

Table 11 - Dry-weight yields of Sorghum almum for 1962.

Plant Introduction Number	Iowa Low seeding rate	Iowa High seeding rate	Minnesota	Missouri
	lbs/a	lbs/a	lbs/a	lbs/a
202410	9089	13505		10380
202411			7026	11196
207837	7441	10382		9146
207838	6250	12864	5621	8737
207840				8716

DISCUSSION

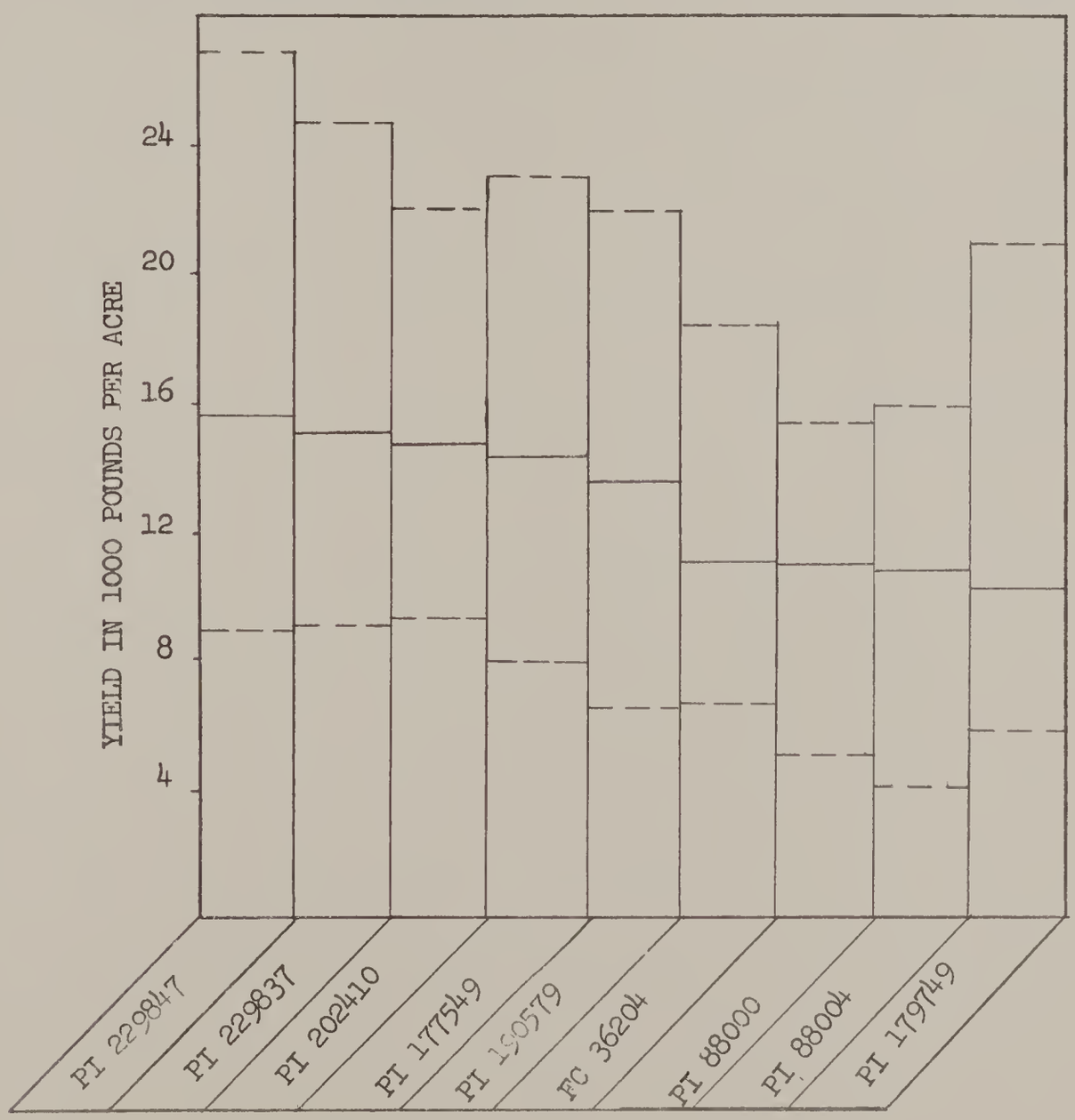
A good pulping sorghum is fibrous, low in solubles and pith content, and has a low leaf percentage. This is in contrast to varietal development of sorghums for forage since succulence, leafiness, low-fiber content, and sweetness are preferred. For both purposes, good standibility, high-yielding capacity, and resistance to diseases and insects are mutually desirable characteristics.

Since pulping results are not available, yields, yield composition, and other characteristics were used to evaluate the crop potential of these sorghums.

In the 1963 tests, the best yields were from Iowa, Indiana, and Georgia. It is evident that, unless supplementary water is available, this group of sorghums has limited crop potential in the Chillicothe area. Also, dry conditions at College Station greatly restricted yields and probably contributed to the low percentage of culms for most test entries.

The over-all yield performance of the nine sorghums is shown in Figure 1. Yieldwise, PI's 229847, 229837, 202410, 177549, and 190579 are the most promising. Of these, the S. almum introductions, 202410 and 190579 are somewhat less desirable. They are quite similar in productivity and composition. The greatest drawback to broomcorn (PI 177549) is its lodging tendency. PI's 229847 and 229837 stand well but are late-maturing, thus seed supply could pose a problem.

Figure 1. The highest, lowest and average yields of sorghums grown at six locations in 1963 (202410 not grown at Chillicothe, Texas).



PI 88000 (kaoliang) has a high leaf percentage and since its over-all yield was poor, it does not warrant further evaluation. In addition, Tift sudan (FC 36204) and the other kaoliang entry (PI 88004) can be removed from further testing. Durra yielded poorly on the average but because of its performance in Iowa, some additional consideration may be merited.

Because of limited seed supply, 1964 activity will be greatly curtailed. Several of the sorghums that were tested in 1963 have excellent yield potentials so studies to determine the proper row-width, plant density, fertilizer rate (especially nitrogen), and harvesting methods are in order as soon as seed is available.

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BOTANICAL COMMENTARY ON NEW SPECIES WITH FAVORABLE POINT RATINGS FOR PULPING POTENTIAL

The new species with favorable pulping potential fall into the families Leguminosae, Malvaceae, Gramineae, Onagraceae, and Compositae. The favorable pulping characteristics of species belonging to the first three families were to be expected on the basis of previous screening experience.

LEGUMINOSAE:

Crotalaria incana L.

Habit: Erect annual herb up to 5 ft. or more in height; branching from the base when growing widely spaced or only in upper one-third when crowded in dense stands.

Distribution and Habitat: According to Senn, this species is widespread in the American tropics, apparently indigenous there and introduced into the Old World. The wild populations observed in Mexico occurred in the States of Puebla and Oaxaca at elevations between 5,000 and 6,000 ft. C. incana has weedy tendencies and was frequently growing on disturbed road margins forming dense stands. Such disturbed open habitats may be typical.

Productivity: In order to estimate the productivity of C. incana, two wild populations were sampled. One stand near Atlixco, Puebla was estimated to produce 6.1 tons of dried plants per acre and another estimate near Cd. Oaxaca indicated 6.4 tons per acre. Under conditions of cultivation, these figures could undoubtedly be greatly increased.

Notes: Although C. incana has already received a favorable rating on the basis of a domestic sample and cannot be regarded as a new species to the screening program, its botanical characteristics are so favorable that comment is warranted. Of the species collected for the pulp screening program in Mexico during the Fall of 1963, C. incana ranks as one of the best prospects encountered. Its erect habit, annual life span and ability to readily colonize disturbed open habitats are favorable characteristics. Furthermore, C. incana produces an abundance of seed which may prove to be of value.

Botanical evaluation: Rating 1.

Crotalaria eriocarpa Benth.

Habit: Erect annual herb reaching 3 ft. or more in height; usually from a single stem and branching in upper portion.

Distribution and Habitat: According to Senn, C. eriocarpa is a Mexican species occurring in the states of Nayarit, Jalisco, Sinaloa, Morelos, Durango, Mexico and Guerrero. Our single collection of this species came from near San Blas, Nayarit at an elevation of approximately 300 ft. in the tropical "tierra caliente". Here it was scattered along road margins and in abandoned fields, occasionally forming dense stands.

Production: No measurements were taken but yields of C. eriocarpa should closely approximate those of C. incana, a species it resembles in habit.

Notes: Like C. incana, this species should receive serious consideration in the new crops program. C. eriocarpa is adapted to a more tropical environment than C. incana but should do well in the southern U. S.

Botanical evaluation: Rating 1 or 2.

Dalea leporina (Ait.) Bull.

Habit: Tall, erect annual herb, reaching a height of 6 ft; branching from the base or only in upper portions when crowded in dense stands.

Distribution and Habitat: According to Kearney & Peebles, D. leporina, is a widely distributed species occurring from New Mexico and Arizona to Guatamala. In Arizona it occurs in mountainous areas from 5,500 to 8,000 ft. In Mexico it is found mainly in the "tierra templada" at elevations of 5,000 ft. or more. Clearly a weedy species readily invading disturbed open habitats such as road margins and abandoned fields.

Productivity: Two wild populations in Mexico were sampled. A roadside stand near San Martin Texmelucan, Puebla yielded an estimate of 5.2 tons of dried plants per acre and another stand near Tepic, Nayarit growing in an abandoned field yielded 5.4 tons per acre. Estimates such as these from wild stands are most encouraging.

Notes: From the botanical standpoint, D. leporina was among the best prospects encountered for the pulp screening program in Mexico during the Fall of 1963. The erect habit, height, annual life span and

weedy nature of this species are favorable characteristics and its wide distribution indicates a considerable range of adaptibility.

Botanical evaluation: Rating 1.

Desmodium nicaraguense Oerst. ex Benth. & Oerst.

Habit: Tall, virgate, woody-stemmed perennial reaching a height of 3 meters.

Distribution and Habitat: Mexico. Our single collection of this species came from near Cd. Oaxaca along the road to Ixtlan de Juarez at an elevation of approximately 5700 ft. Here the plants were scattered on a dry, rocky road bank.

Productivity: No estimates made.

Notes: Desmodium nicaraguense is a perennial but could probably be grown as an annual. Another Mexican Desmodium, D. distortum (Aubl.) Macbr., with habit characteristics much superior to those of D. nicaraguense, was collected for the pulp program in the fall of 1961 by Gentry and Barclay in the vicinity of Tepic, Nayarit. This species has weedy tendencies and was observed to form dense stands along road margins and similar disturbed habitats. If D. distortum has a favorable pulping potential, it should receive careful consideration in the new crops program.

Botanical evaluation: Rating 1.

MALVACEAE:

Sida rhombifolia L.

Habit: Diffuse, single-stemmed annual herb, branching from near the base, attaining a height of 3 ft. or more.

Distribution and Habitat: According to Standley, this species is probably the most common weed of Central America and is widely distributed in the tropics of both hemispheres.

Notes: The erect habit, annual life span, and weedy nature of this species are obvious advantages.

Botanical evaluation: Rating 1 or 2.

GRAMINEAE:

Oxytenanthera abyssinica Munro

Habit: Clump forming, densely tufted; culms up to 40 ft. tall, nearly solid, but usually hollow in the center, ascending or erect.

Distribution and Habitat: Collected in Ethiopia by F. G. Meyer. According to his notes, the species makes an extensive, nearly solid forest that stretches over hilly country for many miles north and south.

Notes: Large member of the Tribe Bambuseae.

Botanical evaluation: Rating 1.

Paspalum arechavaletae Hackel

Habit: Caespitose perennial, 1-2 m. in height. Reaching a height of 1 m. under cultivation at the Jardin Botanico, Facultad de Agronomia, Montevideo.

Distribution and Habitat: South America in the countries of Uruguay, Brazil, Paraguay, and Argentina. A lowland species.

Productivity: Two cuttings in 1963 made on September 20 and May 5 yielded estimates of 13,000 kilograms of dried plants per hectare or approximately 6 tons per acre.

Notes: Paspalum arechavaletae is closely related to both P. quadrifarium and P. exaltatum.

Botanical evaluation: Rating 1.

Paspalum exaltatum Presl

Habit: Densely caespitose perennial, 1.5-2 m. in height. Attaining a height of 1.4 m. under cultivation at the Jardin Botanico, Facultad de Agronomia, Montevideo.

Distribution and Habitat: South America in Uruguay and Argentina. A species of low, humid country.

Productivity: Two cuttings in 1963 made on September 20 and May 2 yielded estimates of 20,000 kilograms of dried plants per hectare or approximately 9 tons per acre.

Notes: Botanists in Uruguay indicate that P. exaltatum is an easy species to cultivate.

Botanical evaluation: Rating 1.

Paspalum haumanii Parodi

Habit: Densely caespitose perennial, 2.5-3 m. in height. Reaching 1.5 m. in height under cultivation at the Jardin Botanico, Facultad de Agronomia, Montevideo.

Distribution and Habitat: South America in the countries of Uruguay, Brazil and Argentina. A species of low, humid country. Characteristic of "los banados de agua dulce" or sweet water marshes.

Productivity: Two cuttings in 1963 made on September 20 and May 5 yielded estimates of 18,000 kilograms of dried plants per hectare or approximately 8 tons per acre.

Notes: Three varieties of P. haumanii occur in Uruguay. This species is closely related to P. exaltatum.

Botanical evaluation: Rating 4 if a true marsh plant.

Paspalum quadrifarium Lam.

Habit: Caespitose perennial, 1 - 1.8 m. in height. Reaching a height of 1.2 m. under cultivation at the Jardin Botanico, Facultad de Agronomia, Montevideo.

Distribution and Habitat: South America in the countries of Uruguay, Brazil, Paraguay, and Argentina. A species of low and humid country.

Productivity: Two cuttings in 1963 made on September 20 and May 5 yielded estimates of 20,000 kilograms of dried plants per hectare or approximately 9 tons per acre.

Notes: According to botanists in Uruguay, this species is used for making roofs on houses.

Botanical evaluation: Rating 1 or 2.

ONAGRACEAE:

Oenothera affinis Cambess

Habit: Stems erect, branching only toward the base, up to 2 m. in height; relatively few leaves.

Distribution and Habitat: Uruguay. Sample cultivated at the Jardin Botanico, Facultad de Agronomia, Montevideo.

Botanical evaluation: Rating 1.

COMPOSITAE:

Eupatorium brevipetiolatum Sch. Bip.

Habit: Shrubby composite reaching 2 m. in height; stems vertical, little branched, leaves only on the extremes; perennial but with vigorous annual growth.

Distribution and Habitat: Uruguay. According to the collectors notes this species is abundant in the highlands.

Productivity: High yield of dry plant material per unit area.

Notes: Easy to cultivate.

Botanical evaluation: Rating 1.

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